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Human Papilloma Virus, Cellular Genetics and Susceptibility to Cervical Cancer

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ABSTRACT Research in relation to the etiology of cervical cancer has made substantial progress in the last two decades both in scientific and operational terms. In many countries, HPV is the most common sexually transmitted infection (STI) and cervical cancer remains the second most common cancer among women worldwide. Although high risk HPV infection has been identified as the primary etiological agent for cervical cancer, various co-factors such as tobacco derived carcinogens, inhalation of air cotaminated through the combustion of coal and kitchen smoke have also been reported to be associated with cervical cancer. Studies on HPV 16 E6 and E7 gene variations provide evidence for the association of specific E6 gene variants with the risk of cervical cancer. While E6 variants may be important in contributing to increased severity of cervical cancer, polymorphisms of other host cellular proteins, such as p53 and p73, may also play an as yet undefined role in modulating the E6-mediated carcinogenic process. Tobacco smoking and chewing has also been found to be associated with increased risk of cervical malignancy. Major classes of carcinogens present in tobacco and tobacco smoke are converted into DNA-reactive metabolites by cytochrome P450 (CYP)-related enzymes, several of which display genetic polymorphism. Individual susceptibility to cancer is likely to be modified by the genotype for enzymes involved in the activation or detoxification of carcinogens in tobacco and repair of DNA damage. Polymorphisms in the carcinogenmetabolizing 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. HPV infection, through the modulation of cellular xenobiotic metabolizing enzymes, may play a role in the ability of cells to handle environmental carcinogens.

INTRODUCTION

Research in relation to the etiology of cervical cancer has made substantial progress in the last two decades both in scientific and operational terms. For decades the epidemiological profile of women with cervical cancer was recognized as suggestive of a sexually transmitted process and several infectious agents were proposed over the years including syphilis, gonorrhea, Chlamydia Trachomatis and type 2 Herpes Simplex Virus. The development of technology to detect the presence of Human Papilloma Virus (HPV) DNA in cellular specimens in the early 1980s made possible the establishment of a definite etiological role for HPV in cervical cancer (Bosch et al. 2002). The association of HPV with cervical cancer has provided the background and the scientific justification for improving screening programs and for developing HPV vaccines (Bosch et al. 2002). In many countries, HPV is the most common sexually transmitted infection (STI) and cervical cancer remains the second most common cancer among women worldwide. In countries where screening is not implemented, cervical cancer is still a major health problem and a frequent cause of death (Bosch et al. 2002). In India, carcinoma of the uterine cervix accounts for about 26% of female cancers, resulting in about 95,000 women developing the disease annually (Jayant et al. 1995). Thus we have 1/6th of the world's population and 1/3rd of the world's cervical cancer burden. Although high risk HPV infection has been identified as the primary etiological agent for cervical cancer, various co-factors such as tobacco derived carcinogens, inhalation of air contaminated through the combustion of coal and kitchen smoke have also been reported to be associated with cervical cancer. Interindividual genetic differences in susceptibility to various carcinogens are important host factors associated with human cancer. It is thus possible that variations in oncogenes, tumor suppressor genes or xenobiotic metabolizing genes may influence cervical carcinogenesis.

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 H 1996). Recognized as sexually transmitted agents, HPVs are believed to be a contributing etiological factor in genital cancers including carcinoma of the uterine cervix. The association between HPV infection and cervical neoplasia has been reported to satisfy all accepted criteria for assessing casuality from epidemiologic studies (Kaufman et al. 1997; Villa 1997; Zehbe and Wilander 1997). 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 (N Munoz 2000). Numerous epidemiological studies indicate a casual relationship between HPV infection and cervical neoplasia (Milde-Langosch et al. 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 (Milde-Langosch et al. 2000, McMurray et al. 2001, Philips et al. 1999). 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 et al. 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). These events are summarized in Figure 1.

HPV Variants

The E6 and E7 genes have been studied in different patient populations and a number of variants have been described (Ginnoudis et al. 2001; Nindl et al. 1999; Xu et al. 2001; Van Duin et al. 2000). 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 et al. 2001). These variants have also been geographically mapped to different areas of the world based on sequence variations of the E6, L1, L2, and long control regions (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. 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 (Pillai et al. 2002).

P53 Polymorphisms

Genetic polymorphism is of significance in cancer because the polymorphic variants are thought to affect any one of the following features in disease progression, i.e., they possess variation in metabolism of carcinogens and drugs, alter risk from exposure, alter effectiveness of therapy, or variation may play a role in disease. While E6 variants may be important in contributing to increased severity of cervical cancer, polymorphisms of other host cellular proteins, such as p53 and p73, may also play an as yet undefined role in modulating the E6-mediated carcinogenic process (Zehbe et al. 2001; Park et al. 2001). P53 is a tumor suppressor gene that responds to genotoxic stress such as DNA damage and hypoxia and is considered the "guardian of the genome" (Lane 1992) and was designated "molecule of the year" in 1993 (Culotta and Koshland 1993). It maintains genomic integrity by arresting cell cycle progression or by inducing apoptosis (Sidransky and Hollstein 1996; Soussi and Beroud 2001). A p53 polymorphism in codon 72 has been described, which encodes either arginine or proline residues. Interestingly, the frequency of the arginine allele increases proportionally to the latitude, while the proline allele shows an inverse effect, i.e., it is more frequent in Black populations, and the arginine allele pre-

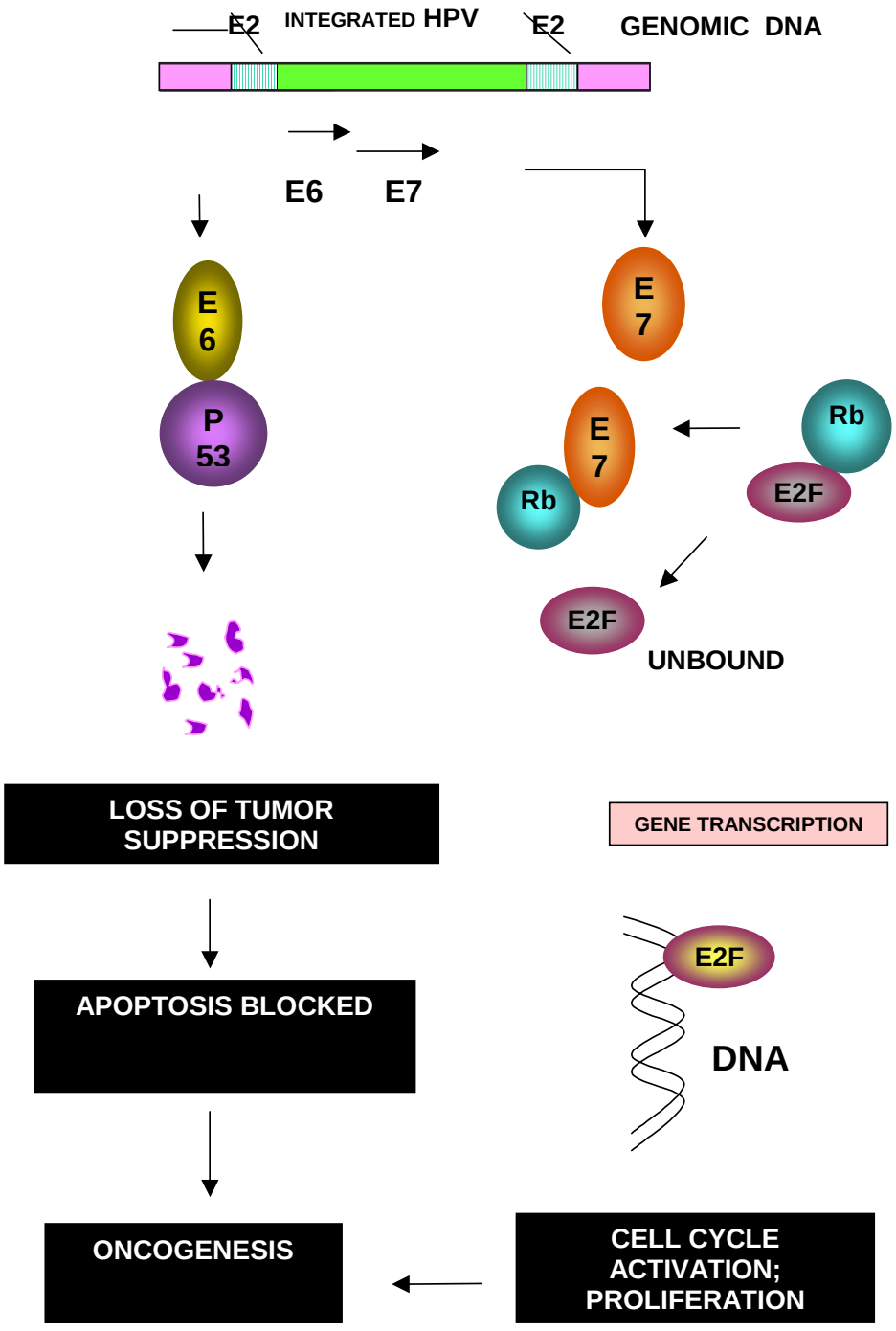

Fig. 1. HPV and Cancer

dominates among Caucasians (Beckman et al. 1994). It has been observed that the p53 arginine variant is more susceptible to HPV E6 mediated degradation than the proline variant. Individuals homozygous for the arginine allele had a 7 times higher chance to bear an HPV associated SCC of the cervix than heterozygous proline/arginine women (Storey et al. 1998). This sensitivity of the Arginine variant could thus be clinically important, as it has been clearly established that p53 degradation is an important feature of HPV associated tumors. However, subsequent analyses performed in the similar and different populations were contradictory making the issue controversial (Rosenthal et al. 1998; Giannoudis et al. 1999; Minaguchi et al. 1998). The two previous pilot studies from India were also contradictory. One report from Western India showed evidence for increased risk of Arginine variant (Saranath et al. 1999), while the other report from Northern India found no evidence for increased risk (Das et al. 1999). Our study, which included various stages of pre-malignant cervical lesions found no evidence of increased risk for progression associated with Arginine homozygosity in any of these subgroups (Pillai et al. 2002).

Tobacco

Tobacco smoking (either active or passive) and chewing has also been found to be associated with increased risk of cervical malignancy (Parazzini et al. 1998; Daling et al. 1996). Nicotine, cotinine and tobacco specific nitosamines have been detected in cervical mucus of smokers and those exposed to tobacco smoke (Prokopczyk et al. 1997; McCann et al. 1992). DNA adducts of aromatic compounds, have been reported to be found with increased frequency in the cervical epithelium of smokers compared to non-smokers (Simons et al. 1993). Reports also showed that women with exposure to wood smoke had a higher risk of cervical neoplasia (Velema et al 2002). Smoking may predispose a woman to the development of cervical cancer by lowering the immune surveillance at the cellular level also (Prokopczyk et al. 1997; Mc Cann et al. 1992). Tobacco use is causally associated with cancers of the lung, larynx, mouth, esophagus, kidneys, urinary tract, and possibly, breast. Major classes of carcinogens present in tobacco and tobacco smoke are converted into DNA-reactive metabolites by cytochrome P450 (CYP)-related enzymes, several of which display genetic polymorphism. Individual susceptibility to cancer is likely to be modified by the genotype for enzymes involved in the activation or detoxification of carcinogens in tobacco and repair of DNA damage.

The mechanism by which PAHs such as B[*a*]P interact with DNA, activate oncogenes, and initiate the carcinogenic process involves the formation of bay-region diolepoxides as the major ultimate carcinogens. B[*a*]P is converted into phenolic metabolites and B[*a*]P-7,8-diol by a CYP-mediated process. Secondary metabolism, mainly involving epoxide hydrolase and other CYP isoforms, leads to the formation of the highly reactive (+)-*anti*-BPDE. Several carcinogens present in tobacco smoke are inactivated by GSTs. The most frequently studied carcinogenic PAH diolepoxide, BPDE, is a relatively good substrate for *GSTM1*, *M2*, and *M3* and better still for *GSTP1* (Bartsch et al. 2000). Because tobacco carcinogens, ROS, and lipid peroxidation products are likely to be substrates for *GSTT1* or *M1*, the extent of DNA damage and ultimately the cancer risk may be affected by polymorphic CYPs and GST detoxifying enzymes (Brockmoller et al. 1998; Nair et al. 1999).

Xenobiotic Metabolizing Enzyme (XME) 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 (eg. 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 catalyze 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. CYP are coded by genes of the CYP super family (Pavanello et al. 2000). Glutathione S-transferases(GSTs) 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 et al. 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 et al. 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 et al. 1995). It has been reported that HPV infection, through the modulation of cellular xenobiotic metabolizing enzymes, may play a role in the ability of cells to handle environmental carcinogens (Chen et al. 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 (H 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 two fold higher catalytic activity compared to the wild type enzyme (H 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 (Merchand et al. 1998). We had earlier reported increased frequency of the m2 polymorphism in oral cancer patients with a long history of tobacco use (T T 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. 2001). 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 to 4.8) and 2.9 (95% Confidence Interval = 1.8 to 4.6) of having a m1 and m2 polymorphism respectively (Pillai 2004, data submitted). 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 to 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 to 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 to 2.5 for GSTM1 and Odds Ratio = 1.7, 95% Confidence Interval = 0.9 to 2.9).

CONCLUSION

It is obvious therefore that the genetic polymorphisms in metabolic enzymes may play a role in development of cervical cancer and more importantly may act as a co-factor in HPV associated carcinogenesis. Cancer occurring in HPV infected tissue can take fifteen to twenty years to develop. During this intervening period, increased chemical carcinogen exposure coupled with inefficient clearing owing to genetic polymorphisms of xenobiotic metabolizing enzymes can be a significant factor. In India, which has a high incidence of HPV associated cancer, as well as in many developing countries the disease is associated with poor socioeconomic conditions. Such women are often exposed to a wide range of carcinogens including those derived from tobacco use, prolonged and sustained inhalation of smoke from kitchen firewood use and possibly

the increased exposure to pesticides from working in the agricultural sector. It is thus clear that large population studies are required in specific populations to determine the biological properties of natural E6 gene variants and eventually to define the molecular mechanisms involved in HPV oncogenecity.

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