Review Article

Impact of Transforming Viruses on Cellular Mutagenesis, Genome Stability, and Cellular Transformation

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It is estimated that 15% of all cancers are etiologically linked to viral infection. Specific cancers including adult T-cell leukemia, hepatocellular carcinoma, and uterine cervical cancer are associated with infection by human T-cell leukemia virus type I, hepatitis B virus, and high-risk human papilloma virus, respectively. In these cancers, genomic instability, a hallmark of multistep cancers, has been explicitly linked to the expression of oncoproteins encoded by these viruses. This review discusses mechanisms utilized by these viral oncoproteins, Tax, HBx, and E6/E7, to mediate genomic instability and cellular transformation. Environ. Mol. Mutagen. 45:304–325, 2005. © 2005 Wiley-Liss, Inc.

Key words: viral transformation; genomic instability; viral-associated cancer; human T-cell leukemia virus type I; hepatitis B virus; human papilloma virus

INTRODUCTION

The World Health Organization estimates that each year approximately 10 million new cancer cases will be reported worldwide and an estimated 6 million people (12% of worldwide deaths) will succumb to some form of this disease (www.who.int/cancer/en). In the United States alone, 2.3 million new cancer cases will be diagnosed and an estimated 556,000 Americans (>1,500 per day) will die each year from cancer [American Cancer Society, 2003]. Worldwide, it has been estimated that 15% of cancer cases are etiologically linked to viral infection, accounting for nearly 1.5 million new cases and 900,000 deaths annually [Butel, 2000].

Viruses have been etiologically linked to specific forms of cancer (Table I), and many other viruses not formally implicated in human tumorigenesis encode proteins capable of immortalizing cells in culture and causing tumors in transgenic animals. As in most cancers, cancers associated with these viruses demonstrate gross chromosomal abnormalities, which have been argued to cause, or at least contribute to, cellular transformation and tumor progression. This review focuses on mechanisms of genomic instability and cellular transformation mediated by the oncoproteins encoded by three well-studied oncogenic viruses: human T-cell leukemia virus type I (HTLV-1), hepatitis B virus (HBV), and human papilloma virus (HPV). These viruses, respectively, are the etiologic agents of adult T-cell leukemia (ATL), hepatocellular carcinoma (HCC), and uterine cervical cancer (UCC).

OVERVIEW OF VIRUSES

Adult T-Cell Leukemia and HTLV-1

HTLV-1 was the first human oncogenic retrovirus discovered [Ploesz et al., 1980] and is now recognized as the etiologic agent of both ATL and a neurodegenerative disease, tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM). HTLV-1 is endemic in parts of Japan, South America, Africa, and the Caribbean and is currently estimated to infect nearly 20 million people worldwide [Franchini, 1995]. Transmission of this virus

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occurs horizontally through the transfer of body fluids (i.e., intravenous drug use, blood transfusion, and sexual contact) and vertically through the ingestion of milk-borne lymphocytes during breastfeeding [Hino et al., 1985; Sugiyama et al., 1986; Wiktorski et al., 1993; Ferreira et al., 1997]. Due in part to the long latency period (2–4 decades), only 4–5% of the infected population are expected to develop ATL, mostly those who were infected as children [Johnson et al., 2001]. Despite the relatively low incidence of ATL, it is a rapidly progressing fatal disease that does not respond to any known treatments and has a mean survival time of 6 months [Jaffé et al., 1984; Shimiyo, et al., 1988].

The HTLV-1 genome encodes several proteins that appear to contribute to infection and oncogenesis. However, numerous studies have established the viral transcriptional transactivator, Tax, as the major viral oncoprotein [Grassmann et al., 1992]. Tanaka et al. [1990] demonstrated that Tax can transform NIH 3T3 and Rat-1 cells and confirmed these observations using mutational analysis. Later Tax alone, and in combination with Ras, was shown to be capable of transforming primary rodent embryo fibroblasts [Pozza et al., 1990; Smith and Greene, 1991]. Tax transgenic mice developed neurofibromas and mesenchymal tumors when Tax was ubiquitously expressed in all tissues, but developed primary peripheral lymphomas comprised of CD8+ T- and NK cells when the Tax transgene was targeted to lymphocytes using the lymphocyte-specific human granzyme B-promoter [Hinrichs et al., 1987; Nerenberg et al., 1987; Grossman et al., 1995]. Although these studies provide strong evidence for the involvement of Tax in tumorigenesis, the exact mechanisms of Tax function have yet to be fully elucidated. Generally, it is believed that Tax promotes tumorigenesis by increasing genomic instability through the disruption of normal host-cell processes, including cellular transcription, DNA repair mechanisms, and cell cycle progression.

### Hepatocellular Carcinoma and HBV

HBV is a double-stranded DNA virus with a ubiquitous worldwide distribution. It is estimated that over 75% of the world’s population lives in regions of high infection [Hollinger and Liang, 2001]. Similar to HTLV-1, HBV is transmitted both horizontally through transfer of body fluids and vertically from mother to child, and 70–90% of mothers chronically infected with HBV will pass the virus on to their child, many of whom will become lifelong carriers. Although the exact mechanism of vertical transmission is not clear, virus-associated antigen has been found in amniotic fluid, cord blood, breast milk, and vaginal secretions, suggesting perinatal rather than transplacental transmission [Chen et al., 2004]. It has been estimated that over 350 million people are chronic carriers of HBV.
and it is believed that over time the infection, potentially in combination with other environmental factors including aflatoxin B ingestion, cigarette smoking, and alcohol consumption, may result in the development of cirrhosis and HCC. In fact, it is currently believed that over 80% of all HCC cases are the result of infection by either HBV or hepatitis C virus (HCV) [Chen et al., 1997].

HCC is the fifth most frequently diagnosed cancer and the fourth leading cause of cancer-related deaths (> 500,000 annually) worldwide. Although the incidence rate in industrialized nations is rather low, due in part to immunization programs, HCC is the leading cause of cancer-related deaths among adults in Southeast Asia and sub-Saharan Africa. Worldwide, the mortality-to-incidence rate is close to or greater than 1, indicating that most patients will not survive longer than 1 year [Hollinger and Liang, 2001].

The HBV genome encodes several proteins required for viral infection and replication. The X-protein (HBx) has been shown to have a cofactor role in HBV-mediated cellular transformation and similar to HTLV-1 Tax, its exact function in oncogenesis remains controversial. Several reports have demonstrated that human HCC tumors, similar to ATL and HTLV-1 Tax, often contain the HBx gene sequence. HCC cells appear to maintain HBx expression throughout all stages of tumorigenesis, unlike ATL cells, which lose Tax protein expression following transformation [Wollersheim et al., 1988; Paterlini et al., 1995; Wei et al., 1995; Su et al., 1998; Sirma et al., 1999]. Although the mere presence of HBx DNA and/or protein in HCC does not prove etiological involvement, studies of HBx expression in cultured cells and transgenic animals support a role for HBx in cellular transformation.

Early studies demonstrated that HBx is able to transform SV40-immortalized rodent cells both in culture and in nude mice [Hohne et al., 1990; Gottlob et al., 1998; Yu et al., 1999]. Several HBx transgenic mouse lines have been generated and demonstrate accelerated liver tumor formation [Kim et al., 1991; Yu et al., 1999]. Studies of other HBx transgenic lines, however, have reported that HBx alone does not stimulate tumorigenesis but rather sensitizes liver cells to environmental carcinogens, including diethylnitrosamine and aflatoxin B [Dragani et al., 1990; Slagle et al., 1996]. Furthermore, bitransgenic mice overexpressing HBx and c-Myc develop liver tumors at an accelerated rate, suggesting that HBx, like Tax, cooperates with cellular protooncogenes to transform cells [Terradillos et al., 1997; Lakhatakia et al., 2003]. Although these data do not delineate the mechanisms of HBx-mediated cellular transformation, they do demonstrate that HBx enhances HBV-induced tumorigenesis. Similar to Tax, it is believed that HBx mediates transformation by increasing genomic instability and mutation frequency through dysregulation of cellular processes.

**Uterine Cervical Cancer and HPV**

HPV is one of the most common sexually transmitted diseases (STDs) worldwide and is believed to be the most prevalent STD in the United States. Despite the fact that genital HPV infection is not a reported disease, it is estimated that between 1 and 5.5 million new cases occur each year, and the overall prevalence of infection is greater than 20 million people in the United States alone [Cates, 1999]. HPV is most often transmitted by direct contact between two epithelial surfaces and is facilitated in part by the presence of abrasions or lesions [Oriel, 1971; Moy et al., 1989; Euvrard et al., 1993]. The incidence of HPV infection correlates directly with the number of sexual partners an individual has encountered and infections are rare in sexually inexperienced and/or young women [Rosenfeld et al., 1989; Moscicki et al., 1990; Fairley et al., 1992; Bauer et al., 1993; Andersson-Ellstrom et al., 1994; Gutman et al., 1994; Rylander et al., 1994].

Over 100 different papilloma virus family members exist and, in humans, are responsible for a variety of benign proliferations, including warts, epithelial cysts, intraepithelial neoplasia, anogenital, orolaryngeal, and oropharyngeal papillomas, keratoacanthomas, and other types of hyperkeratoses. Infection with specific high-risk HPVs, including HPV-16 and -18, is associated with approximately 90–99% of uterine cervical cancers (HPV-16, specifically, accounts for 50–60% of positive biopsies; HPV-18, 10–20%) and more than 50% of other anogenital tumors, including cancers of the vagina, vulva, penis, and anus, as well as a small percentage of head and neck tumors [zur Hausen, 1996]. In fact, numerous studies have demonstrated that the association between infection with high-risk HPV and uterine cervical cancer is greater than the association between cigarette smoking and lung cancer [Burd, 2003]. Uterine cervical cancer in adult women is the fourth most diagnosed form of cancer in the United States and the most commonly diagnosed form in developing countries, where it accounts for approximately 25% of all female cancers [Harro et al., 2001; Jemal et al., 2004]. Furthermore, cervical cancer ranks second to breast cancer as the leading cause of female cancer-related deaths worldwide [Jin et al., 1999]. Despite these facts, the development of UCC from persistent high-risk HPV infection occurs only in a minority of infected women and, like cancers related to HTLV-1 and HBV, requires a long latency period, suggesting that the accumulation of mutations within the host genome and other predisposing or environmental factors are required for transformation [Duensing and Munger, 2004].

HPVs are small double-stranded DNA viruses that encode several proteins, two of which, E6 and E7, are essential for HPV-mediated cellular transformation. Like HTLV-1 and HBV, the exact mechanisms of HPV-mediated transformation have not been fully elucidated.
Several reports have determined that, similar to HTLV-1 and HBV and their associated cancers, the HPV-16 and -18 genomes can be isolated from cervical cancer biopsies [Durst et al., 1983; Boshart et al., 1984]. Furthermore, several groups have reported the presence of the E6 and E7 proteins in all stages of HPV-positive cancers cells, suggesting that these proteins are required for both the initiation and progression of tumorigenesis [zur Hausen, 1996].

Although it is not entirely clear what role the E6 and E7 proteins play in these processes, these proteins, despite their independent oncogenic properties, cooperate in mediating cellular transformation [Munger et al., 1989]. Expression of E6 alone is sufficient to induce anchorage-independent growth of NIH 3T3 cells and immortalize human mammary epithelial cells, while expression of E7 by itself leads to transformation of NIH 3T3 cells and, at a very low efficiency, to immortalization of the natural host cell, human keratinocytes [Munger et al., 1989; Band et al., 1990; Sedman et al., 1991; Storey and Banks, 1993; Kiyono et al., 1998; Liu et al., 1999; Riley et al., 2003]. Coexpression of E6 and E7 efficiently transforms human keratinocytes [Hawley-Nelson et al., 1989; Munger et al., 1989]. Interestingly, in contrast to Tax- or HBx-transformed cells, E6-, E7-, or E6/E7-transformed cells rarely produce tumors when transplanted into nude mice [Kaur and McDougall, 1988; Pirisi et al., 1988; Woodworth et al., 1988]. However, numerous versions of E6, E7, and E6/E7 transgenic mice develop either benign tumors or cancers [Kondoh et al., 1991; Arbeit et al., 1993, 1994; Griep et al., 1993; Lambert et al., 1993; Searle et al., 1994; Comerford et al., 1995; Herber et al., 1996; Song et al., 1999]. The frequency and type of malignancy seems to depend largely on the promoter used to drive expression of the viral oncogene(s). Furthermore, a recent report utilizing chronic estrogen administration with E6, E7, or E6/E7 transgenic mice demonstrated that E7 was sufficient to produce high-grade cervical dysplasia and invasive cervical malignancies, while mice expressing E6 alone only developed low-grade cervical dysplasia and did not show new neoplastic progression. In double-transgenic mice, the E6 protein enhanced the malignant phenotype produced by E7, resulting in larger and more extensive cervical cancers. Since these observations depended on chronic estrogen administration, the cooperative transforming effects of E6 and E7 appear to require additional stimuli [Riley et al., 2003]. In fact, HPV-infected women who smoke cigarettes, take oral contraceptives, are overweight, or have been exposed to other STDs are more likely to develop UCC than those infected women who have not been exposed to these additional risk factors [Daling et al., 1996; Kjellberg et al., 2000].

Taken together, these studies demonstrate that E6 and E7 are necessary, but not sufficient, for HPV-mediated cellular transformation. Although these data are fairly conclusive, the exact mechanisms leading to transformation by these oncoproteins have not been fully elucidated. However, similar to Tax and HBx, it is known that E6 and E7 affect a variety of cellular processes including cell cycle progression, cellular transcription, and the DNA-damage response.

**VIRALLY MEDIATED GENOMIC INSTABILITY**

Chromosomal aberrations and genomic instability play a major role in the development and progression of multi-step cancers, including ATL, HCC, and UCC. In fact, it has been argued that a series of five to six independent genetic events is required for healthy cells to become transformed [Fearon and Vogelstein, 1990; Hanahan and Weinberg, 2000]. The four types of genetic alterations that typically occur in tumors include single or small nucleotide sequence changes, altered chromosomal numbers, chromosomal translocations, and gene amplifications [Lengauer et al., 1998]. It is not surprising that each of these modifications has been identified in HTLV-1-related ATL, HBV-mediated HCC, and HPV-dependent UCC, as well as a number of other virally mediated cancers. Genomic changes associated with these virus-associated cancers have been explicitly linked to the expression of each of the viral-specific oncoproteins.

Single or small nucleotide changes involving base substitutions, deletions, or insertion of one to a few nucleotides can alter the amino acid composition, and presumably function, of a protein. Unlike other types of mutations, nucleotide changes cannot be detected through cytogenetic analysis. Instead, investigators rely on functional and sequence analysis of genically integrated reporter genes to estimate the overall cellular mutation frequency and spectrum. Although a number of cellular genes isolated from ATL, HCC, and UCC tumors are mutated, little evidence links these specific mutations to the expression of Tax, HBx, or E6/E7. However, several studies have reported that Tax, E6, and HBx are each associated with increased mutation frequencies within the cellular genome.

Miyake et al. [1999] demonstrated that Tax expression in Big Blue Rat-2 cells (BBR-2; Stratagene, La Jolla, CA), which contain a genomically integrated lacI gene, resulted in a 2.8-fold increase in mutation frequency compared to non-Tax-expressing cells. Additional studies by these and other investigators showed that exposure of BBR-2 cells to other mutagens (i.e., ultraviolet light, ethynitrosourea, dimethylbenz[a]anthracene) at levels capable of transforming cells resulted in mutation frequencies similar to those in Tax-expressing cells [Manjanatha et al., 1996; Zimmer et al., 1996]. These results suggest that the increased mutation frequency observed in the presence of Tax may be physiologically important in the process of transformation.
Increased mutations frequencies have also been associated with the expression of *high-risk* HPV E6 but not *low-risk* HPV E6 or E7 [Havre et al., 1995]. Cells expressing *high-risk* E6 protein demonstrated a fivefold increase in spontaneous mutations when compared to control cells and a twofold increase when compared to *low-risk* HPV E6-expressing cells. Additionally, sequence analysis of the *Hprrt* gene showed a dramatic increase in deletions, rearrangements, and point mutations in the presence of *high-risk* E6. Since these changes were prevalent in cells expressing *high-risk* but not *low-risk* E6, these data suggest that increases in mutation frequency contribute to cellular transformation.

Although studies to determine mutation frequency in HBx-expressing cells have not been reported, double-transgenic mice expressing HBx and possessing an integrated λ transgene reporter have been used to determine whether HBx enhances the accumulation of spontaneous mutations in the livers of these animals. These studies demonstrated that HBx alone does not increase the rate of spontaneous mutations; however, when these mice were treated with aflatoxin B, a 24% increase in overall mutation frequency was observed [Madden et al., 2000, 2002]. Sequence analysis of the transgene demonstrated an altered mutation spectrum with a twofold increase in G:C→A:T transversions in response to aflatoxin B treatment. Together, these reports suggest that HBx is not sufficient for tumor development, but rather enhances the effects of, or sensitizes cells to, other mutagens.

Alterations in chromosome number are found in all types of tumors and are designated as either aneuploidy, the gain or loss of whole chromosomes, or loss of heterozygosity (LOH), the gain or loss of chromosome sections or single genes [Lengauer et al., 1998]. Cytogenetic and microsatellite analysis of ATL, HCC, and UCC tumors, as well as some virally infected or transformed cells, has demonstrated both aneuploidy and LOH. These changes are thought to alter the expression or function of cellular oncogenes, tumor suppressors, and/or other essential proteins.

ATL cells express a number of random chromosomal anomalies. Although no uniform karyotypic abnormalities have been consistently identified in all tumors [Kao et al., 2000], specific abnormalities including trisomy 3 and 7, amplification of 14q [also commonly identified in Epstein-Barr virus (EBV)-associated Burkitt’s lymphoma], deletions of 1q and 6q, monosomy of the X chromosome, and loss of the Y chromosome are more prevalent than others in ATL cells [Zeich et al., 1976; McCaw et al., 1977; Fukuhara and Rowley, 1978; Ueshima et al., 1981; Miyamoto et al., 1983; Rowley et al., 1984; Whang-Peng et al., 1985, 1993; Sanada et al., 1987; Fujita et al., 1989; Kamada et al., 1992]. Loss of certain regions such as chromosome 17p is especially interesting because it is also commonly deleted in HCC tumors and contains the *p53* gene [Hatta et al., 1998].

Several reports have investigated the chromosomal integrity of HCC cells and have identified deletions in portions of chromosomes 1p, 6q, 7q, 8p, 13q, 16p, and 17p and LOH at regions containing *p53* (17p13), *RB* (13q14), *AXIN1* (16p13), and a number of other cellular genes that are commonly affected in HBV-positive HCC tumors [Murakami et al., 1991; Boige et al., 1997; Nagai et al., 1997; Satoh et al., 2000]. Portions of amplified chromosomal regions that are also frequently identified in HCC include regions encoding the *c-MYC* oncogene at 1q and other cellular oncogenes present at 3q, 6p, 7p, 8q, and 17q [Levy et al., 2002]. Thus, the amplification of proto-oncogenes coupled with partial or complete loss of tumor suppressor genes most likely contributes to HBV-mediated cellular transformation and tumor progression.

Of the three cancer types reviewed here, HPV-positive UCCs demonstrate the longest list of gross chromosomal rearrangements. Several investigators have reported partial or complete loss of chromosomes 1p, 2q, 3p, 4p and q, 5q, 6q, 7q, 8p, 9p and q, 11q, 12q, 13q (including the *RB* gene), 14q, 17p and q, 18q, and 19p and q in a variety of combinations. LOH has also been reported at a number of other sites, including 3p, 4p and q, 5p, 6p and q, and 11q. Similar to ATL and HCC cells, UCC cells demonstrate a gain of chromosomes 1q, 3p, 5p, and 8q, which include regions encoding cellular proto-oncogenes, such as the *c-MYC* gene (1q) [Mitra, 1999; Buendia, 2002; Rao et al., 2004]. Thus, the spectrum of chromosomal rearrangements found in UCC, ATL, and HCC likely cause or contribute to cellular transformation and tumor progression.

Micronuclei (MN), which are produced as by-products of DNA damage, are small nuclei-like bodies found outside of the nucleus. As a result of treatment with aneuploidogenic agents, cells produce MN with whole chromosomes or centric chromosomal fragments, whereas cells treated with clastogenic compounds produce MN containing acentric fragments [Majone et al., 1992]. MN provide a measure of a cell’s ability to repair damaged DNA and segregate chromosomes after exposure to specific mutagens. Consistent with observations of chromosomal abnormalities in ATL, HCC, and UCC tumors, several researchers have demonstrated an increased prevalence of MN in Tax, HBx, and EBV LMP-1 immortalized or transformed cells.

Majone et al. [1993] investigated MN in HTLV-1-transformed cells using wild-type Tax, transcriptionally active Tax mutants, and transcriptionally inactive Tax mutants. Their results demonstrated a 1.5–1.7% frequency of MN in cells that express wild-type Tax or transcriptionally active Tax mutants compared to MN frequencies of only 0.4–0.7% when the transcriptionally inactive Tax mutants were analyzed. Additionally, these authors report an increase in kinetochore-positive and -negative MN, demonstrating both clastogenic and aneuploidogenic effects of Tax [Majone et al., 1993; Majone and Jeang, 2000].
Together, these data suggest that Tax-mediated overexpression of certain cellular genes coupled with transcriptional repression of other genes represses DNA repair while enabling DNA replication and cell cycle progression in the presence of mutagen-induced DNA lesions. Replication of these unrepairled lesions may potentially lead to the accumulation of mutations and contribute to Tax-mediated genomic instability.

In addition to Tax-mediated MN formation, several other viral oncoproteins have been investigated for their ability to induce MN in transformed cells. EBV LMP-1-expressing epithelial cells were recently shown to contain increased numbers of both spontaneous and bleomycin-induced MN [Liu et al., 2004]. Furthermore, Liezey et al. [2002] reported nearly a threefold increase in MN in HBx-expressing cells compared to HBx-negative control cells. Although there have been no reports of MN associated with HPV infection or expression of its oncoproteins, MN formation may be a shared mechanism among viral oncoproteins and should be investigated more thoroughly.

In addition to increased MN in HBx-expressing cells, these cells also demonstrated an increase in chromosomal translocations when compared to HBx-null cells [Lievezey et al., 2002]. Chromosomal translocations are fusions of different chromosomes or of noncontiguous segments of a single chromosome. As a result, two deleterious outcomes are possible. First, the fusion can place a specifically regulated gene under the control of an improperly regulated promoter, leading to altered expression of the protein. Second, a fusion between two genes can occur, resulting in the formation of a novel protein with an unknown and potentially oncogenic function [Lengauer et al., 1998]. These chromosomal abnormalities often result from the improper repair of DNA damage. Since many viral oncoproteins repress DNA repair mechanisms, it is not surprising that viral-associated cancers demonstrate these abnormalities [Savage, 2002]. Similar to HBx, HPV E6- and E7-transformed epithelial cells, as well as SV40 T-antigen-transformed cells, also demonstrate extensive duplications and translocations, the former involving chromosomal aberrations at regions 11q13q22;q23 [Hoffschir et al., 1988; Sutherland et al., 1988; Ray et al., 1990; Macoska et al., 2000]. Furthermore, sister chromosome translocation at the 11q loci has been observed in an EBV-transformed lymphoblastoma cell line [Seki et al., 1992]. Despite the fact that this genetic abnormality is common in viral-associated tumors and in cells transformed by viral oncoproteins, the exact mechanisms by which chromosomal translocations contribute to tumorigenesis remain to be fully defined.

It is well recognized that gene amplification occurs at a greater rate in cancer cells than in normal cells; however, very little is known about the mechanism of gene amplification [Lengauer et al., 1998]. It is believed that amplifications occur in response to chromosomal breaks that result from incorrectly resolved DNA recombination [Kuo et al., 1994; Wettergren et al., 1994; Coquelle et al., 1997]. These abnormalities are most prevalent when p53 is inactivated in mammalian cells [Yin et al., 1992]. The ability of viral oncoproteins including Tax, HBx, and E6/E7 to repress or functionally inactivate p53 and certain DNA repair mechanisms suggests that these viral oncoproteins may contribute to gene amplification.

Although few published studies have directly investigated the potential effects of viral oncoproteins on gene amplification, increased gene amplification in Tax-expressing cells and a possible mechanistic explanation for these observations have been recently reported by our research group [Lemoine and Marriott, 2002]. Using the PALA [N-(phosphonomethyl)-1-aspartate] assay, which measures amplification of the trifunctional enzyme, CAD (carbamyl phosphate synthetase/aspartate transcarbamylase/dihydroorotate), we demonstrated a four- to fivefold increase in gene amplification in the presence of Tax. In addition, cells failed to undergo a typical p53-dependent, PALA-mediated G1 arrest in the presence of Tax, suggesting that the ability of Tax to alter PALA-dependent cell cycle arrest depends on its ability to repress p53 activity [Lemoine and Marriott, 2002]. Although the effect of viral oncoproteins on gene amplification remains poorly defined, this evidence combined with an overall understanding of factors that contribute to gene amplification (loss of p53 function and DNA repair mechanisms) predicts that other viral oncoproteins may also contribute to this form of genomic instability.

Thus far, we have reviewed data that support a role of the viral oncoproteins Tax, HBx, and HPV E6/E7 in mediating cellular transformation and, in some cases, tumor progression in ATL, HCC, and UCC, respectively. Increasing evidence suggests that expression of these viral oncoproteins is associated with chromosomal abnormalities found in their respective cancers, including point mutations, altered chromosome numbers, chromosomal translocations, and gene amplifications. Although exact mechanisms of virus-mediated transformation are not fully understood, viral oncoproteins are known to affect a number of common cellular mechanisms that are likely to be involved in cellular transformation and in some cases tumor progression. These mechanisms include altered cellular transcription and cell cycle progression, as well as repression of tumor suppressor genes and DNA repair pathways (Table II).

TRANSCRIPTION MODULATION BY VIRAL ONCOPROTEINS

Although mutations are probably the most important cellular event leading to neoplastic transformation, oncogenic viruses have evolved ways to alter host gene
### TABLE II. Summary of the Effects of Viral Oncoproteins on Cellular Processes

<table>
<thead>
<tr>
<th>Oncoprotein</th>
<th>Transcription</th>
<th>Cell cycle</th>
<th>Tumor suppressor proteins</th>
<th>DNA repair</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tax</td>
<td>Activates/expresses through CREB/ATF, SRF, and NF-κB pathways; can also interact with and affect the function of basal transcription factors</td>
<td>Stimulates bypass of G1/S and G2/M checkpoints; disrupts the mitotic spindle checkpoint</td>
<td>Inactivates p53 and pRB</td>
<td>Inhibits NER, BER, and may affect double-stranded break repair</td>
</tr>
<tr>
<td>HBx</td>
<td>Activates transcription through cis elements (XRE), which include binding sites for AP-1, AP-2, NF-κB, SRF, c/EBP, ETS, ATF-1, and CREB; HBx may also interact with basal transcription factors</td>
<td>Stimulates bypass of G1/S and G2/M checkpoints</td>
<td>Inactivates p53 and pRB</td>
<td>Inhibits NER</td>
</tr>
<tr>
<td>E6/E7</td>
<td>Interferes with signaling pathways and may have a global affect on transcription by altering the function of basal transcription factors (TBP and TAF-110)</td>
<td>Overrides cell cycle controls; disrupts mitosis</td>
<td>Inactivates p53 and pRB</td>
<td>Inhibits NER</td>
</tr>
</tbody>
</table>

expression without changing gene structure. Generally, complex viruses encode one or several nonstructural transcription regulators that ensure optimal viral gene expression and dictate profound changes in host gene expression to create a favorable cellular environment for virus replication. Viral oncoproteins can affect transcription by binding directly to the transcription machinery, interacting with specific transcription factors, or influencing upstream signaling pathways. All of these mechanisms are employed by Tax, HBx, and HPV E6/E7 to modulate cellular transcription pathways and are believed to contribute to the transforming properties of these oncoproteins by interfering with cellular mechanisms that prevent the accumulation of mutations or limit cell proliferation in the presence of DNA damage.

Tax was originally described as a potent transactivator of the HTLV-1 long terminal repeat whose effect was mediated by three copies of a cyclic AMP response element (CRE) located within the viral promoter [Sadowski et al., 1984]. The transcriptional activity of Tax affects four discrete transcription factor pathways: CREB/ATF [Franklin et al., 1993; Adya et al., 1994; Low et al., 1994; Adya and Giam, 1995], NF-κB [Ballard et al., 1988; Leung and Nabel, 1988; Ruben et al., 1988; Kanno et al., 1994; Sun et al., 1994], SRF [Fujii et al., 1991, 1992], and AP-1 [Armstrong et al., 1993; Fujii et al., 2000; Mori et al., 2000]. By exploiting these pathways, Tax modulates the expression of more than 50 cellular genes, including growth factors and their receptors, cytokines, transcription factors, cell cycle control proteins, DNA repair proteins, and cell adhesion molecules, which collectively contribute to cellular transformation [Lemoine et al., 2001].

Like Tax, HBx is a promiscuous transcriptional modulator that can activate transcription of HBV as well as a wide variety of cellular promoters through cis elements, generically designated XREs. These elements include binding sites for AP-1, AP-2, NF-κB, SRF, c/EBP, ETS, ATF1, and CREB [Murakami, 1999]. Although an interaction between HBx and some of these cellular transcription factors has been well documented, the majority, including AP-1, NF-κB, and SRF, do not appear to bind HBx. In these latter cases, the transactivation mechanism is believed to rely on more general processes such as modulation of signaling cascades and interaction of HBx with the basal transcriptional machinery. Although HPV E6 and E7 are not primarily considered to be transcription modulators, they can interfere with several cellular signaling pathways leading to altered function of transcription factors such as NF-κB and AP-1. HPV E7 can also interact with transcription coactivators and several basal transcription factors, including the TATA binding protein (TBP) and TBP-associated factor-110 (TAF-110), suggesting that it may have a global effect on cellular transcription [Mazzarelli et al., 1995; Massimi et al., 1996; Phillips and Vousden, 1997].

Cyclic AMP (cAMP)-responsive element-binding factor (CREB) and its closely related orthologs, activating transcription factor-1 (ATF-1) and the cyclic AMP response element modulator (CREM), belong to the basic leucine zipper (bZip) family of transcription factors and may additionally alter their DNA binding specificity. They respond to increased levels of the secondary messenger cAMP through phosphorylation induced by protein kinase A (PKA) [Gonzalez and Montminy, 1989]. PKA phosphorylation of CREB at serine 133 recruit the coactivators p300/CBP to the CRE, thereby activating transcription [Chrivita et al., 1993; Arias et al., 1994]. Both Tax and HBx are able to bypass the normal cAMP signaling pathway by interacting with CREB and CBP, resulting in transcriptional activation in a phosphorylation-independent manner [Harrod et al., 1998; Van Orden and Nyborg, 2000; Pfum et al., 2001]. Furthermore, Tax and HBx can increase DNA binding and dimerization and may alter the specificity of several bZip transcription factors. For example, it is well established that Tax enhances CREB-dependant transactivation of the imperfect viral CRE, but not the consensus
certain probabilistically such as the p100, which is instability and inhibition of transcriptional repression, and leads to profound perturbations in cell cycle progression and apoptosis. Through the CREB/ATF pathway, Tax upregulates the expression of BCL-xl, an antiapoptotic factor, and cyclin D2, which has a role in cell cycle progression [Santiago et al., 1999; Tsukahara et al., 1999]. In contrast, Tax represses the cyclin A and cyclin D3 genes through a CRE by an unknown mechanism that is believed to involve the formation of different complexes on the respective promoters [Kibler and Jeang, 2001]. This may affect cell cycle progression through S-phase, as well as mitotic exit. Other genes, including ICAM-1, fucosyltransferase, and galectin-3, are upregulated by Tax through CRE elements and have been shown to play an important role in leukemic cell infiltration and metastasis [Hsu et al., 1996; Tanaka et al., 1996; Hiraiwa et al., 2003]. Interestingly, HBx also upregulates galectin-3 expression resulting in increased metastatic properties of liver tumors [Hsu et al., 1999]. NF-IL6, another bZip family member, mediates HBx transactivation of the IL-8 gene in human hepatoma cells, probably providing the transformed cells with a continuous autocrine growth stimulus [Mahe et al., 1991].

In addition to activating gene expression, the interaction of Tax with CBP is proposed to have inhibitory effects on the transcription of certain cellular promoters. Jeang et al. [1990] first described repression of the DNA polymerase β gene by Tax and identified an E-box DNA element responsible for this effect. It was subsequently reported that p53, p18/\textit{BNK4}, and, more recently, the human telomerase (\textit{hTERT}) promoters, all of which contain the E-box recognition sequence, are repressed by Tax [Akagi et al., 1995; Suzuki et al., 1999b; Gabet et al., 2003]. It is hypothesized that this repression results from direct competition between Tax and E-box binding transcription factors of the β helix-loop-helix family for recruitment of CBP/p300 [Suzuki et al., 1999b]. Tax expression was found to diminish the binding of p53 to CBP, an interaction that was previously shown to be required for enhanced p53-mediated transcription. This effect is probably also due to competitive binding of Tax to CBP and contributes to the inhibition of p53 transactivating function by Tax [Ariumi et al., 2000]. HBx can also repress p53 transcription through an E-box; however, the exact mechanism remains to be identified [Lee and Rho, 2000]. The interaction of HBx with p53 also appears to play an important role in the functional inactivation of this tumor suppressor by inhibiting p53 sequence-specific DNA binding and transactivating ability [Feitelson et al., 1993; Wang et al., 1994]. In addition, HBx exerts direct inhibitory effects on the transcription of the p21 gene, independently of p53, suggesting that it is able to prevent cell cycle arrest by targeting a critical factor downstream of p53 [Ahn et al., 2001]. Lastly, the HPV E6 oncoprotein has the ability to bind CBP/p300 and disrupt CBP-mediated acetylation of p53, thereby reducing the DNA binding affinity of p53 and its transactivation potential [Gu and Roeder, 1997; Patel et al., 1999]. Taken together, these data suggest that viral oncoproteins have evolved different ways to inhibit the expression of certain critical genes involved in cell cycle control and DNA repair, ultimately leading to unchecked cell proliferation and genome instability.

NF-κB signaling is a common target of viral infection. In resting cells, NF-κB proteins are bound and maintained in the cytoplasm by inhibitory partners, such as IkBα, IkBβ, p100, and p105 [Chen and Greene, 2004]. Cellular stimulation and activation of cellular signaling pathways induce phosphorylation of the inhibitory proteins and target them for ubiquitination and subsequent degradation. As a result, the nuclear localization sequence of NF-κB is unmasked, and the active form translocates to the nucleus, where it activates target genes. HTLV-1-infected cells, as well as cells expressing Tax, induce and maintain an activated NF-κB pathway. Tax directly interacts with the inhibitory proteins IkBα and IkBβ, as well as the IKKγ subunit of the kinase regulatory complex, collectively leading to disruption of inactive cytoplasmic NF-κB complexes and nuclear translocation of active NF-κB [Hirai et al., 1992; Beraud et al., 1994; Kanno et al., 1994; Suzuki et al., 1995]. Exploiting the NF-κB pathway, Tax activates a large number of cellular genes that promote lymphocyte growth, activation, and survival. These genes fall into several categories, including cytokines and growth factors such as IL-1, IL-2, IL-6, IL-8, IL-15, and TGFB, growth factor receptors such as IL-2Rα and OX40, and antiapoptotic factors such as BCL-xl [Lemoine et al., 2001]. Special importance is attributed to Tax activation of the cellular proto-oncogene, c-MYC, which can independently mediate cellular transformation via NF-κB regulation [Keath et al., 1984; Adams et al., 1985]. Additionally, NF-κB-dependent stimulation of both IL-2 and IL-2 receptor expression is thought to create an autocrine feedback loop that can stimulate growth factor-independent proliferation of infected cells in vivo [Ballard et al., 1988; Wano et al., 1988].

HBx also usurps cell signaling pathways to activate NF-κB- and AP-1-dependent transcription [Murakami, 1999]. Direct interactions between HBx and components of the MAPK, SAPK, and PKC signaling pathways have been demonstrated, and it has been speculated that HBx may act as an adaptor or kinase activator to enhance the phosphorylation of associated proteins [Cross et al., 1993; Kekule et al., 1993; Diao et al., 2001]. Additional
mechanisms of NF-kB activation by HBx include direct interaction with inhibitory proteins, such as the p105 precursor and IKKz, and induction of oxidative stress by mitochondrial-associated HBx protein [Su and Schneider, 1996; Waris et al., 2001]. Regardless of the mechanism, HBx induction of NF-kB and AP-1 activity ultimately leads to the acceleration of cell cycle progression, enhanced proliferation, and may contribute to repression of apoptosis.

As an additional mechanism to induce cell proliferation, Tax binds to serum response factor (SRF) and induces aberrant expression of several immediate early nuclear oncogenes, including c-FOS, Fos-related-antigen-1 (FRA-1), early growth response gene-1 (EGR-1) and EGR-2 [Alexandre and Verrier, 1991; Fujii et al., 1992, 1995; Suzuki et al., 1993]. Recruitment of CBP to these cellular promoters is required for Tax activation [Shuh and Derse, 2000]. It was recently reported that HBx directly interacts with EGR-2 and EGR-3 and enhances their interaction with CBP to activate FAS ligand gene expression in hepatoma cells [Yoo et al., 2004]. This effect was proposed to confer an immune-privileged status to the tumor, promoting cell proliferation in vivo.

The data reviewed here described various mechanisms used by viral oncoproteins to manipulate cellular transcription pathways in order to alter cellular gene expression. While changes in transcription do not directly lead to cellular transformation, they can induce a highly proliferative state, which is generally thought to be a sine qua non for oncogenesis. In addition, these changes can affect the capacity of the host cell to respond properly to genotoxic agents by altering the efficient cellular concentration of proteins involved in repair of DNA damage, cell cycle control, and apoptosis, ultimately inducing genomic instability and promoting oncogenic transformation.

**DYSREGULATION OF CELL CYCLE PROGRESSION**

Mammalian cells have evolved mechanisms to maintain genomic integrity by inducing cell cycle arrest in response to DNA damage. Such checkpoint mechanisms allow time to repair DNA damage before the cell commits to DNA replication and cell division. Through the function of their oncoproteins, viruses have developed several strategies to subvert key regulatory factors in order to promote DNA replication and cellular proliferation (Fig. 1). Reprogramming of the cell cycle may benefit the virus by promoting efficient replication of the viral genome in the host cell. Viral oncoproteins mediate this reprogramming by abrogating negative growth signals and checkpoint controls. As a result, viral oncoproteins contribute to the proliferation and accumulation of cells that are genetically unstable, some of which may contain transforming mutations.

Cell cycle progression is controlled by interplay between positive and negative regulators. One mechanism that viral oncoproteins use to override cell cycle control is association with cyclin and cyclin-dependent kinase (cyclin-CDK) complexes and/or molecules that regulate their activities, termed cyclin-dependent kinase inhibitors (CKIs). During G1, D-type cyclins (D1, D2, D3) form active complexes with CDK4 or CDK6. These activated cyclin D-CDK4/6 complexes phosphorylate the retinoblastoma protein (pRB), causing it to dissociate from E2F. The active E2F protein then transcribes genes necessary for S-phase entry [Adams, 2001]. These genes include those that encode cyclins E and A, two essential mediators of the G1-S transition in mammalian cells [Weinberg, 1995]. Tax has been shown to interact with CDK4 and CDK6 and enhance their binding to cyclin D2. This results in earlier onset of cyclin D-CDK4/6 kinase activity followed by an accumulation of hyperphosphorylated pRB in Tax-expressing cells compared to cells not expressing Tax [Haller et al., 2002]. As a result, E2F is released in Tax-expressing cells early in G1, shortening the length of G1 and accelerating S-phase entry [Lemoine and Marriott, 2001].

HBx has also been shown to stimulate cell cycle progression by accelerating transit through the G1/S and G2/M checkpoints [Benn and Schneider, 1995]. Compared with serum stimulation, HBx strongly increases the rate and level of CDK2 and CDC2 kinase activation and their respective formation of active complexes with cyclins A and E or cyclin B [Koike et al., 1994]. HBx also causes cyclin D1 overexpression, which is maintained throughout the cell cycle [Klein et al., 2003]. In addition, analysis of temperature-sensitive mutants demonstrated that HBx activates the cyclin A promoter via its ATF/CREB transcription factor binding site and that this activation depends on the Src (RAS-RAF-MAPK) pathway [Bouchard et al., 2001]. Studies have also reported the interaction of HBx with the RPB5 subunit of RNA polymerase [Cheong et al., 1995] as well as the interactions of HBx or Tax with TBP [Caron et al., 1993; Qadri et al., 1995]. These observations raise the possibility that direct modulation of transcription factors by Tax and HBx can contribute to cell cycle deregulation.

The HPV E6 and E7 oncoproteins also override cell cycle controls to induce cellular hyperproliferation. Similar to HBx, HPV E7 is able to activate transcription from the cyclin A and E promoters. Transient transfection assays showed that activation of cyclin A and E transcription by E7 is indirect and requires the synthesis of additional cellular gene products [Benn and Schneider, 1995; Bouchard et al., 2001]. E7 also interacts indirectly with cyclin E-CDK2, mediated through p107, a pRB-related protein [McIntyre et al., 1996]. Interestingly, E7 was shown to downregulate cyclin D1 expression and, in the presence of E6, was shown to disrupt cyclin D1-CDK4
Fig. 1. Effects of viral oncoproteins Tax, HBx, and E6/E7 on cell cycle progression following DNA damage. Following DNA damage, cell cycle progression is halted by the activation of negative regulators of cyclin/CDK complexes (black lines and arrows). The viral oncoproteins Tax, HBx, and E6/E7 have evolved mechanisms to subvert these negative regulators (red lines) or activate positive regulators (green arrows) in order to promote cell cycle progression and subsequently promote DNA replication and cellular proliferation in the presence of DNA damage. The mechanisms utilized by these oncoproteins include inhibition of the tumor suppressor proteins p53 and pRB as well as the cyclin-dependent kinase inhibitors p21\(^{Waf1/Cip1}\), p27\(^{Kip1}\), p16\(^{Nk4a}\), p15\(^{Nk4b}\), p18\(^{Nk4c}\), and p19\(^{Nk4d}\), and the mitotic-phase regulators MAD-1 and -2. In addition to preventing the functions of cell cycle checkpoint proteins, the viral oncoproteins also activate cyclin/CDK complexes to stimulate cell cycle progression.

complexes [Xiong et al., 1996]. This is unlike the functions of Tax and HBx, which stimulate cyclin D-CDK kinase activities during G1. It is possible that E7 downregulates cyclin D-CDK activity in order to shorten the length of G1 so that cells progress more rapidly into S-phase. In support of this theory, the E7/cyclin E/CDK2 complex exhibited kinase activity throughout the cell cycle with a significant peak in S-phase [McIntyre et al., 1996]. Thus, inhibition of G1-specific CDK activity and induction of S-specific CDK activity may be a mechanism by which HPV promotes DNA replication in the presence of antiproliferative signals.

The enzymatic activities of cyclin-CDK complexes are controlled by CKIs, including p21\(^{Waf1/Cip1}\), p27\(^{Kip1}\), p15\(^{Nk4b}\), p16\(^{Nk4a}\), p18\(^{Nk4c}\), and p19\(^{Nk4d}\) [Peter and Hershkowitz, 1994; Morgan, 1995; Sherr and Roberts, 1995]. Since these inhibitors can cause cell cycle arrest by regulating cyclin-CDK kinase activities, CKIs are recognized as tumor suppressor proteins and are frequently deleted in human cancer cells [Sherr and Roberts, 1995; Sherr, 2004]. Thus, the functional inhibition of CKIs by viral oncoproteins may allow these viruses to mimic the effects of deleting these genes in cancer cells in order to promote constitutive activation of cyclin-CDK complexes and cell cycle progression.

Tax has been shown to bind p15\(^{Nk4b}\) and p16\(^{Nk4a}\) and block the inhibitory effects of these CKIs on cyclin D-CDK4 activity [Suzuki et al., 1996, 1999a; Low et al., 1997; Iwanaga et al., 2001; Hatta and Koeffler, 2002]. This would reverse p15\(^{Nk4b}\) and/or p16\(^{Nk4a}\)-induced G1 growth arrest and remove barriers that normally restrict replication of damaged DNA. As previously discussed, Tax represses transcription through E-box elements, including those in the p18\(^{Nk4c}\) and p19\(^{Nk4d}\) promoters.
This correlates with a marked reduction of p18\textsuperscript{INK-4c} and p19\textsuperscript{INK-4d} mRNA levels in HTLV-1-infected T-cells [Suzuki et al., 1999a]. Thus, these observations indicate that Tax can suppress the activity of INK4 family members through two independent mechanisms: by functional inhibition via direct protein-protein binding and by repression of transcription. These effects may play roles in HTLV-1-induced deregulation of the cell cycle, thereby promoting cellular transformation by Tax. In contrast, numerous studies have reported the activation of p21\textsuperscript{Waf1/Cip1} expression by Tax [Akagi et al., 1996; Cereseto et al., 1996; De La Fuente et al., 2000; Hatta and Koeffler, 2002]. This observation was unexpected since it is well known that p21\textsuperscript{Waf1/Cip1} inhibits DNA replication and allows cells to repair DNA damage [Fotedar et al., 2004]. Thus, stimulation of p21\textsuperscript{Waf1/Cip1} by Tax suggests that this traditional inhibitor may have an alternate function that may be required for cellular transformation by Tax. p21\textsuperscript{Waf1/Cip1} stimulates the NF-κB pathway [Joyce et al., 2001], which functions in cellular transformation by Tax. Recent studies report that p21\textsuperscript{Waf1/Cip1} is involved in stabilization and transport of active cyclin D-CDK4 kinase complexes [Haller et al., 2002]. It is possible that stimulation of p21\textsuperscript{Waf1/Cip1} expression by Tax could increase the steady-state levels of active cyclin D-CDK4 and enhance its phosphorylation of pRB. This would describe another role for p21\textsuperscript{Waf1/Cip1} in Tax-mediated transformation and explain the upregulation of p21\textsuperscript{Waf1/Cip1} in Tax-expressing cells.

Studies of HPV E6 and E7 proteins show that these viral oncoproteins can also activate the function of CKIs and increase the proliferative potential of cells. The E7 protein binds and inactivates p21\textsuperscript{Waf1/Cip1} and p27\textsuperscript{Kip1} [Funk et al., 1997; Jones et al., 1997], reversing the inhibitory effects of these CKIs on cyclin A-CDK2 and cyclin E-CDK2, respectively [Martin et al., 1998; Schulze et al., 1998]. In other studies, transient transfection assays show that E6 expression dramatically decreases the level of p21 mRNA [Li et al., 1994b; Xiong et al., 1996], suggesting that this CKI may also be transcriptionally inhibited by HPV.

Similar to the mechanism used by E6, HBx regulates p21\textsuperscript{Waf1/Cip1} on a transcriptional level; however, the precise mechanism of this regulation remains controversial [Kwun and Jang, 2004]. Additionally, the results of recent studies suggest that epigenetic alterations through methylation may be a mechanism by which HBV transcriptionally regulates tumor suppressor proteins. Interestingly, methylation of the p16\textsuperscript{INK-4a} promoter was detected in 50% of HBV-positive HCC cases, but was absent in all noncancerous tissues examined [Narimatsu et al., 2004]. Analogous results were obtained when similar studies were performed on the p15\textsuperscript{INK-4b} promoter [Yang et al., 2003]. Whether or not aberrant promoter methylation can be attributed to HBx activity remains to be seen.

**EFFECTS OF VIRAL ONCOPROTEINS ON TUMOR SUPPRESSOR PROTEINS**

Transforming viruses can deregulate cell cycle progression by overriding growth suppressive functions of the p53 tumor suppressor and retinoblastoma (pRB) proteins. pRB and its related proteins p107 and p130 play important roles in regulating E2F-mediated transcription. In its hypophosphorylated form, pRb forms a protein-protein complex with E2F and inhibits its transcriptional activity [Weinberg, 1995; Sherr, 2004]. Activation of cyclin D-CDK kinase complexes during G1/S-phase of the cell cycle leads to hyperphosphorylation of pRB and subsequent release of active E2F [Matsushima et al., 1994]. This results in transcriptional activation of E2F-responsive genes, which promote cell cycle progression into S-phase. The E7 protein of high-risk HPV has been shown to bind the hypophosphorylated form of pRB, disrupting the ability of pRB to bind and inactivate E2F [Pagano et al., 1992]. Once bound, E7 destabilizes pRB by inducing its degradation via a ubiquitin-proteosome pathway [Boyer et al., 1996]. However, recent studies have shown that E7 may confer acute biological effects that are independent of its ability to inactivate pRB. Using a Cre-lox system to abrogate pRB expression in the epidermis of transgenic mice, Balsitis et al. [2003] showed that epidermis lacking pRB displayed epithelial hyperplasia, aberrant DNA synthesis, and improper differentiation. Remarkably, mouse epidermis deleted in pRB and expressing E7 showed increased hyperplasia and dysplasia, indicating that other activities besides E7’s inactivation of pRB must contribute to the biological properties of E7 in vivo. Indeed, E7 has been reported to bind many different cellular proteins in addition to pRB. These include the pRB-related proteins p107 and p130 [Dyson et al., 2003]. In transient transfection assays, E7 was shown to decrease steady-state levels and half-lives of p107 and p130 [Gonzalez et al., 2001]. Like pRB, p107 and p130 bind to and modulate the function of E2F family members [Mulligan and Jacks, 1998]; thus, in a pRB-deficient environment, it is possible that E7 may retain the ability to dysregulate the cell cycle and DNA synthesis through inactivation of p107 and p130. As mentioned above, E7 can also bind and inactivate CDK inhibitors p21 and p27 [Funk et al., 1997; Jones et al., 1997], thus providing another mechanism through which E7 can disrupt these cellular processes. Because of the multifunctional nature of E7, its role in transformation remains unclear.

HTLV-1 Tax has also been shown to overcome pRB-mediated cell cycle arrest; however, unlike E7, Tax does so indirectly by affecting events upstream of pRB. Tax is believed to regulate pRB phosphorylation by stimulating cyclin D-associated kinase activities. In experiments utilizing a Tax-inducible cell line, Tax expression caused a 50% increase in pRB phosphorylation when compared to
cells not expressing Tax [Neuveut et al., 1998; Haller et al., 2002]. This increase in pRB phosphorylation correlated with a sevenfold increase in cyclin D-associated kinase activity as well as with activation of E2F-responsive genes. Tax induction also caused faster progression of cells into S-phase when compared to cells not expressing Tax. Moreover, Tax has been shown to associate directly with cyclin D3, and this interaction resulted in increased cyclin D3-associated kinase activity in vitro [Neuveut et al., 1998]. Finally, Tax can bind and inactivate p16(INK4a), removing a negative regulator of cyclin D activity [Suzuki et al., 1996; Low et al., 1997]. Taken together, these data suggest that Tax subverts cyclin D, an upstream regulator of pRB, through multiple mechanisms. Like Tax, HBx does not bind pRB [Farshid et al., 1997], and recent evidence suggests that HBx may also function upstream of pRB by causing cyclin D1 overexpression and increasing intranuclear cyclin D1 accumulation when expressed in mammary epithelial cells [Klein et al., 2003].

In mammalian cells, p53 protein is crucial for maintaining genome integrity by controlling the cell's response to a variety of stresses such as DNA damage and oncogene activation. p53 participates in several cell cycle checkpoints and is upregulated during the DNA damage response. As a consequence of its pivotal role in guarding against cellular transformation, p53 is a major target for inactivation by viral oncoproteins. The E6 protein of high-risk HPV interacts directly with p53 to cause its inactivation. E6 induces rapid proapoptotic degradation of p53 by interacting with the host-cell protein E6-AP, an E3 ubiquitin ligase [Scheffner et al., 1990, 1993]. The E6/E6-AP enzymatic complex then binds to p53 and mediates the conjugation of ubiquitin moieties to p53, targeting it for proteolytic degradation [Scheffner et al., 1993]. As a consequence, cells expressing E6 do not accumulate high levels of p53 in response to DNA damage and, as a result, fail to induce the G1/S cell cycle checkpoint properly. Recent evidence showed that p53 degradation mediated by E6 and disruption of RB family member functions mediated by E7 are both required to induce polyplody in human keratinocytes [Patel et al., 2004]. In this study, polyplody was not caused by failure in cytokinesis, but rather from a disruption in mitosis. The authors hypothesized that the mitotic failure may have been the result of deregulation of G2-M phase genes by E6 and E7 proteins. In microarray analyses, a number of genes involved in the G2-M transition of the cell cycle upregulated in E6/E7-expressing cells, including PLK1, a serine/threonine kinase known to regulate several events involved in mitotic exit and in events leading to cytokinesis [Patel et al., 2004]. Similar to the induction of polyplody, upregulation of PLK1 protein in E6/E7 cells was dependent on the ability of E6 mutants to degrade p53 and on the ability of E7 mutants to bind and repress pRB. Thus, the abnormal chromosomal segregation observed in E6- and E7-expressing cells could in part be due to the deregulation of this kinase, the events of which seem to require the cooperative functions of both E6 and E7 proteins.

Although several groups have documented a direct interaction between p53 and HBx, the functional significance of this interaction is not fully understood. As previously discussed, HBx binding to p53 inhibits the sequence-specific DNA binding and transcriptional activating properties of p53 [Feitelson et al., 1993; Elmore et al., 1997; Lin et al., 1997]. This interaction also alters formation of the p53 preinitiation transcription complex [Wang et al., 1994]. Additional studies showed that HBx inhibits the interaction between p53 and several DNA repair proteins [Lee et al., 1995; Wang et al., 1995b, 1996]. Several reports also demonstrated an inhibitory effect of HBx on p53-mediated apoptosis, although this remains controversial [Wang et al., 1995a; Elmore et al., 1997; Huo et al., 2001]. The inhibition of apoptosis by HBx correlated with sequestration of p53 in the cytoplasm, which was not detected in HBx-null hepatocytes [Ueda et al., 1995]. This result suggests that decreased nuclear p53 levels in HBx-expressing cells may inhibit apoptosis and contribute to hepatocellular carcinogenesis.

Unlike E6/E7 and HBx, HTLV-1 Tax does not bind p53 [Yamato et al., 1993; Pise-Masison et al., 1998]. However, Tax does have the ability to abrogate the transactivating function of p53 and override p53-mediated cell cycle arrest or apoptosis in HTLV-1-infected cells [Uittenbogaard et al., 1995; Pise-Masison et al., 1998; Ariumi et al., 2000]. One mechanism by which Tax inhibits p53 function involves differential phosphorylation of p53 via the p65/RELA subunit of NF-kB, which could alter the interactions of p53 with a variety of transcription factors [Jeong et al., 2004]. Tax, as discussed above, also suppresses p53-mediated transactivation by competing with p53 for binding to the transcriptional coactivator CBP/p300, thereby disrupting p53-dependent G1 arrest or apoptosis [Grossman, 2001]. The inhibition of p53-dependent cellular genes blocks induction of the G1/S checkpoint [Van Orden et al., 1999; Ariumi et al., 2000]. As a consequence, DNA replication proceeds even in the presence of negative growth-suppressive signals. Unchecked cell cycle progression resulting from continued proliferative signals might then lead to immortalization and cellular transformation.

**IMPAIRMENT OF CELLULAR DNA REPAIR PATHWAYS BY VIRAL ONCOPROTEINS**

Although no common type of chromosomal damage has been associated with the development of ATL, HTLV-1-transformed lymphocytes isolated from patients...
or immortalized in cell culture demonstrate a large variety of chromosomal aberrations, including deletions, duplications, translocations, rearrangements, and aneuploidy [Miyamoto et al., 1983; Rowley et al., 1984; Itayomi et al., 1990]. Tax expression interferes with various processes involved in DNA metabolism, normal progression through the cell cycle, and apoptotic elimination of cells containing dangerous amounts of DNA damage. However, Tax itself does not directly induce DNA lesions [Saggio et al., 1994]. DNA alterations resulting from exogenous genotoxic factors or normal replication processes are corrected by several repair pathways, including nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), homologous recombination (HR), and nonhomologous end joining (NHEJ) [Sancar et al., 2004]. Suppression of some of these pathways by Tax may increase the occurrence of mutations, potentially contributing to cellular transformation.

The first indication that Tax expression may affect DNA repair came with the finding that Tax can repress transcription of DNA polymerase β, an essential enzyme involved in BER [Jeang et al., 1990]. BER removes a wide variety of genomic lesions, including hydrolytic DNA depurination, deamination of cytosine and 5-methylcytosine, reaction products of hydroxyl-free radicals, and covalent DNA adducts [Wood and Shivji, 1997]. Two distinct BER mechanisms correct these specific types of lesions. Short-patch repair involves single nucleotide filling after removal of individual altered nucleotides, whereas long-patch repair resolves DNA segments up to 10 nucleotides in length. DNA polymerase β is only required for short-patch repair, the major BER pathway [Matsumoto and Kim, 1995]. As a consequence, HTLV-1-transformed cells, as well as cells transiently expressing Tax, have a defect in short-patch BER [Philippot and Buehring, 1999]. Interestingly, the HPV oncoprotein E6 has also been proposed to alter the function of DNA polymerase β. A recent study demonstrated that HPV E6 interacts with XRCC1, a key factor involved in single-strand DNA-break repair [Ifnner et al., 2002]. Binding of E6 to XRCC1 has a negative effect on BER, probably by displacing DNA polymerase β from the active damage repair complex. The influence of this event on the viral life cycle is not known, but it may increase availability of the DNA polymerase required for replication of the viral genome in terminally differentiated keratinocytes.

Possibly the most versatile repair pathway, NER is a target of Tax, HBx, and the HPV oncoproteins. NER detects helix distortions resulting from ultraviolet irradiation and carcinogenic agents and recognizes transcription complexes stalled at DNA lesions. This repair pathway requires DNA polymerases δ and ε and uses PCNA as a cofactor [Riedl et al., 2003]. When genomic lesions are encountered, elevated levels of the cyclin-dependent kinase p21\textsuperscript{waf1/Cip1} interact with PCNA, blocking DNA replication without affecting the PCNA-dependent repair. However, excessive PCNA can overcame the p21\textsuperscript{waf1/Cip1}-induced replication block, allowing the polymerase to synthesize DNA through template lesions, resulting in nucleotide misincorporation [Li et al., 1994a]. Interestingly, the ability of Tax to suppress NER has been directly related to its ability to activate the PCNA promoter transcriptionally, resulting in elevated levels of PCNA protein [Lemoine et al., 2000].

Several studies demonstrating that HBx expression inhibits cellular DNA repair pathways showed that unscheduled DNA synthesis and host-cell reactivation of UV-damaged reporter genes are compromised by HBx [Becker et al., 1998; Groisman et al., 1999; Jia et al., 1999]. The increased rate of liver tumor development in HBx transgenic mice exposed to chemical carcinogens is consistent with HBx inhibition of hepatocyte DNA repair [Slagle et al., 1996]. Inhibitory effects of HBx on NER are thought to be mediated by direct interaction of HBx with UV-damaged DNA as well as with several proteins involved in cellular DNA repair. A yeast-two-hybrid approach was used to identify a cellular protein, XAP-1 (HBx-associated protein 1), as the human homologue of the simian UV-damaged DNA-binding protein (UV-DDB) [Lee et al., 1995]. Later, this interaction was shown to inhibit the host-cell NER pathway [Becker et al., 1998]. These authors proposed that the binding of HBx to XAP-1 and UV-damaged DNA inhibits the first step of NER, which involves recognition of DNA lesions. Since the HBV genome must be converted from partially double-stranded DNA to covalently closed circular DNA (cccDNA) by an unknown host mechanism, they also speculated that these effects of HBx on the host DNA repair machinery might ensure correct processing of the viral genome and sustain viral replication. More recent work has demonstrated that the binding of HBx to UV-DDB is indispensable for productive viral infection and the transactivation ability of HBx [Sitterlin et al., 2000].

In addition to targeting UV-DDB, HBx uses at least two additional mechanisms to inhibit NER. p53 plays an important role in transcription-coupled DNA repair, and its interactions with the XBP and XPD helicases of the multisubunit transcription factor TFIIH are required for this function. It is believed that the association of p53 with TFIIH normally inhibits trans-lesion replication and initiates the assembly of repair complexes at the damaged sites [Wang et al., 1995b]. HBx blocks the interaction of p53 with XBP and XPD, effectively inhibiting p53-dependent repair [Prost et al., 1998]. Second, in experiments performed in cells containing a mutant version of p53, HBx was shown to inhibit NER in a p53-independent mechanism by interacting directly with XBP and XPD to enhance the helicase activity of the TFIIH complex [Jia et al., 1999]. Functional p53 is thought to inhibit the helicase activity of this complex,
thereby enabling the assembly of repair complexes [Qadri et al., 1996].

While gross chromosomal aberrations are the most common type of genomic alterations found in HPV-infected cells, high-risk HPV E6 and E7 oncoproteins also interfere with specific DNA repair pathways resulting in single or small nucleotide changes. A limited number of studies have investigated the effect of HPV oncoproteins on NER. Work done in primary keratinocytes immortalized with HPV16 found increased mutation frequencies in both the transcribed and untranscribed strands of a transgene, demonstrating a failure to repair UV-induced DNA damage and identifying a defect in general genome repair and transcription-coupled NER [Rey et al., 1999]. The mechanism of this defect is still unknown, but does not appear to involve the viral oncoprotein E6, which was not detected in the system used by the authors. In contrast, Wani et al. [2002] showed that expression of E6 but not E7 inhibited the removal of bulky DNA adducts by depleting cellular p53, thereby antagonizing its function in global genome repair. The E6 protein was also shown to be responsible for degradation of O6-methylguanine-DNA methyltransferase (MGMT), an enzyme that functions in a stochiometric reaction to reverse DNA lesions produced by endogenous mutagens and alkylating agents [Srivenugopal and Ali-Osman, 2002]. Together, these data suggest that HPV infection induces a mutator phenotype that is at least partially due to E6-mediated degradation of DNA repair proteins, which may contribute to virally induced transformation.

Currently, there is no indication that the viral oncoproteins discussed in this review affect recombinational repair. However, several lines of evidence suggest that Tax may interfere with the repair of DNA double-strand breaks. As previously discussed, Tax-expressing cells form MN containing whole chromosomes, as well as centric and acentric fragments, indicating the presence of unresolved chromosomal breaks [Majone et al., 1993]. Secondly, in situ labeling of DNA ends showed that HTLV-1-transformed cells as well as HeLa cells expressing Tax had increased numbers of unprotected DNA ends when compared to controls [Majone and Jeang, 2000]. Recent findings demonstrating that Tax can repress the expression of the human telomerase gene (hTERT) has been suggested as a possible explanation for these observations, since DNA ends are stabilized by the transient addition of telomeric repeats. Inhibition of telomerase activity would also foster persistent telomere dysfunction, thereby allowing an accumulation of unbalanced chromosomal rearrangements [Gabet et al., 2003]. A more recent study presents contrasting evidence for the activation of hTERT transcription by Tax in primary T-lymphocytes infected with HTLV-1 in vitro, as well as in ex vivo ATL samples. The authors proposed a model in which Tax inhibits hTERT expression in mitogen-stimulated cells through an E-box DNA element, but activates its transcription in resting cells in an NF-kB-dependent manner [Sinha-Datta et al., 2004]. It has been postulated that the interference of Tax with induction of hTERT would result in a transient state of genetic instability that is replaced by induction of telomerase transcription in unstimulated cells to promote long-term proliferation and prevent telomere attrition. Similarly, HPV E6 activates hTERT expression by an unknown mechanism, which is probably required to maintain telomere length in the rapidly dividing neoplastic cells [Veldman et al., 2001].

As discussed in the previous sections, aneuploidy is a general characteristic of cancer cells and is commonly found in ATL, HCC, and UCC. Alterations in chromosome number arise as a result of mitotic perturbations and are generally considered to be the cause, rather than a consequence, of transformation [Rasnick, 2002]. To ensure the correct partitioning of replicated chromosomes during mitosis, cells use the mitotic spindle assembly checkpoint (MSC) as a surveillance mechanism to arrest the progression from metaphase to anaphase in the presence of spindle damage or segregation errors. Several genes identified in genetic screens have been shown to contribute to proper function of the MSC complex, including monopolar spindle 1 (MPS1), budding uninhibited by benzimidazole (BUB) genes, and mitotic arrest-deficient (MAD) 1, 2, and 3 [Jallepalli and Lengauer, 2001]. Several HTLV-1-transformed cell lines are deficient in MSC integrity following exposure to nocodazole [Kasai et al., 2002]. Consistent with this observation, Tax binds and inactivates hsMAD1 and sequesters both MAD1 and -2 in the cytoplasm, preventing the latter from properly localizing at the kinetochore [Jin et al., 1998]. Therefore, the ability of Tax to disable an important protective mechanism that protects against chromosomal mis-segregation during mitosis provides a potential mechanism for the high incidence of aneuploidy in ATL cells.

Chromosomal defects resulting from multipolar mitoses have long been recognized as a hallmark of high-risk HPV-associated cervical lesions. Such aberrant cell divisions are prompted by an abnormal number of mitotic spindle poles formed by the centrosomes. Initial observations of keratinocytes expressing high-risk HPV E6 and E7 showed that the two oncoproteins cooperate to induce supernumerary centrosomes [Duensing and Munger, 2001, 2002]. Remarkably, this effect has not been observed with low-risk HPV E6 or E7. High-risk HPV E7 can quickly subvert the control of centrosome duplication during mitosis, leading to the assembly of multipolar spindles and ultimately to aneuploidy [Duensing and Munger, 2001]. This function of E7 is independent of its ability to bind and inactivate pRB but appears to depend on a domain that mediates its interaction with p21Cip1/Waf1. Therefore, alterations in cyclin/CDK2 complexes have been proposed.
as a mechanism of E7-induced centrosome duplication errors [Duensing and Munger, 2003]. Interestingly, the proportion of multipolar E7-expressing cells in metaphase is much larger than in later mitotic phases, suggesting that the mitotic checkpoint is activated and generally prevents the completion of aberrant divisions.

SUMMARY

This review has analyzed mechanisms that lead to increased genome instability and cellular transformation mediated by the viral oncoproteins of HTLV-1, HBV, and HPV, three well-studied viruses that are the etiologic agents of ATL, HCC, and UCC, respectively. Expression of their viral oncoproteins, Tax, HBx, and E6/E7, correlates with the formation of specific types of genomic alterations often associated with these cancers. Convincing evidence demonstrates increased point mutations, altered chromosome numbers, chromosomal translocations, and gene amplification in cells transformed by or transiently expressing these proteins. Although the mechanisms these viral oncoproteins use to transform cells and, in some cases, promote tumor progression have not been fully delineated, we have reviewed the known pathways that regulate these activities and discussed the effects of viral oncoproteins on these pathways. As we have discussed, each of these three viral oncoproteins affects a number of cellular processes. Although they do not necessarily affect the same pathways or affect them in the same way, the overall mechanisms appear to be highly analogous among viruses and may represent commonly utilized pathways in genome instability and cellular transformation.

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