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Effect of Transforming Viruses on Molecular Mechanisms Associated With Cancer

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Abstract

Viruses have been linked to approximately 20% of all human tumors worldwide. These transforming viruses encode viral oncoproteins that interact with cellular proteins to enhance viral replication. The transcriptional and post-transcriptional effects of these viral oncoproteins ultimately result in cellular transformation. Historically, viral research has been vital to the discovery of oncogenes and tumor suppressors with more current research aiding in unraveling some mechanisms of carcinogenesis. Interestingly, since transforming viruses affect some of the same pathways that are dysregulated in human cancers, their study enhances our understanding of the multistep process of tumorigenesis. This review will examine the cellular mechanisms targeted by oncogenic human viruses and the processes by which these effects contribute to transformation. In particular, we will focus on three transforming viruses, human T-cell leukemia virus type-I, hepatitis B virus and human papillomavirus. These viruses all encode specific oncogenes that promote cell cycle progression, inhibit DNA damage checkpoint responses and prevent programmed cell death in an effort to promote viral propagation. While the transforming properties of these viruses are probably unintended consequences of replication strategies, they provide excellent systems in which to study cancer development.

Cancerous cells arise from a multistep accumulation of genetic alterations. Genetic abnormalities contribute to tumor development through a succession of changes some of which confer an increased probability of gaining additional genetic changes. Hanahan and Weinberg (2000) described six hallmarks of cancer that can arise as a result of genetic aberrations. These include proliferation independent of growth signals, insensitivity to anti-growth signals, escape from apoptosis, invasion of other tissues, limitless replicative potential and development of angiogenic abilities.

According to the World Health Organization (WHO) about 20% of all cancers worldwide result from chronic infections, such as those brought about by viruses. DNA viruses such as Epstein Barr virus (EBV), hepatitis B virus (HBV), human papilloma virus (HPV) and human herpes virus type 8 (HHV-8) have been linked to the development of lymphomas, liver cancer, cervical carcinomas, and Kaposi's Sarcoma, respectively. However, only two

retroviruses, human T-cell leukemia virus type I (HTLV-1) and HIV, have been associated with human cancers. While genetic alterations are the predominant mechanism of oncogenesis, viruses have evolved additional methods to affect the same critical pathways in an attempt to promote viral replication. Specifically, some transforming viruses can manipulate host cell processes to promote cellular proliferation in the absence of environmental growth cues, inhibit cellular responses to anti-growth signals, and provide resistance to apoptosis. Interestingly, these viral effects mirror three of Hanahan and Weinberg's six hallmarks of cancer. This review will focus on some of the molecular mechanisms used by HTLV-1, HPV and HBV to hijack the host cell machinery, ultimately leading to carcinogenesis.

HTLV-1 is the only human retrovirus that has been shown to be the direct etiological agent of a human malignancy, adult T-cell leukemia (ATL) (Poiesz et al., 1980). An estimated 10–20 million people worldwide are infected with HTLV-1, but only a small fraction (<5%) of infected individuals develop ATL (Edlich et al., 2000). The HTLV-1 genome encodes the typical retroviral enzymatic and structural genes *gag*, *pol* and *env*, but also encodes a unique 40-kDa oncoprotein, Tax. Tax is a transcriptional transactivator of viral gene expression, doing so through interactions with cellular transcription factors and co-activators (Kashanchi and Brady, 2005). Although Tax has no cellular homologue, both cell culture and animal model systems have shown that Tax expression is sufficient to transform cells and induce tumors, thus establishing its role as an oncoprotein (Grassmann et al., 1989; Tanaka et al., 1990; Grossman et al., 1995).

HPV is a double stranded DNA virus that infects keratinocytes. The HPV life cycle follows the differentiation program of the infected cell. In cells from the basal layer of the epithelia, the virus is present in low copy numbers, however cellular differentiation leads to increased viral copy number and its integration into the host genome (Munger et al., 2004). Over 100 HPV genotypes have been categorized as either low risk or high risk for development of cancer. Viral infection most commonly manifests as skin warts or papillomas, but high risk HPV types are causative agents of uterine cervical cancer, which accounts for about 12% of all cancers in women (zur Hausen, 2002). The 13 high risk HPVs are sexually transmitted and have been associated with cervical, anal, vulvar and penile cancers (Munger et al., 2004). HPVs express two major oncoproteins, E6 and E7, and the E6 and E7 proteins of high risk HPVs are most highly associated with transformation. Individually, E6 and E7 can transform cells in culture, but with less efficiency than when expressed together (Munger et al., 1989; zur Hausen, 2002).

HBV is also a DNA virus, but is unusual in that it has a partially double stranded DNA genome. The virus primarily infects liver cells or hepatocytes. Chronic HBV infection plays an important role in the development of liver cirrhosis and hepatocellular carcinoma (HCC), one of the most common cancers worldwide. The viral genome encodes four major proteins: C, X, P, and S. Expression of the 17-kDa transcriptional regulator X viral oncoprotein (HBx) is one of the major risk factors for developing HCC. Similar to Tax and E6/E7, HBx can transform primary cells in culture (Hohne et al., 1990; Gottlob et al., 1998b), however HBx expression alone is not always sufficient to induce tumor formation in animal models. Mice expressing the X gene under the control of the human alpha-1-antitrypsin regulatory

element have only minor pathological liver alterations, however when exposed to a hepatocarcinogen they display a twofold increase in the incidence of hepatocellular carcinoma (Slagle et al., 1996). HBx expressing animals are therefore more sensitized to environmental carcinogens and could be more susceptible to developing chromosomal abnormalities.

All three of these viruses encode proteins that reprogram host cell machinery to promote viral replication. An essential part of viral replication and survival is to enhance cellular proliferation and prevent cell death. Here we will discuss viral mechanisms that promote cellular proliferation through evasion of apoptosis, deregulation of the cell cycle, and inhibition of cell cycle checkpoints and DNA repair pathways.

Enhancing Cellular Proliferation

One of the hallmarks of transformed cells, the ability to proliferate in the absence of external growth stimuli, generally results from defects in specific components of the cell cycle machinery. Cell cycle progression from G1 through S, and G2 into mitosis, is directed through a sophisticated interplay of positive and negative regulatory signals. A cell will not commit to DNA replication until it has received appropriate mitogenic signals indicating an ideal environment for proliferation and cell survival. Normal progression through the cell cycle is regulated by sequential activation of cyclin and cyclin-dependent kinase (CDK) complexes. CDKs are catalytically activated by binding to specific cyclin subunits: cyclin D in G1, cyclins E and A in S phase and cyclins B and A in mitosis. Active cyclin-CDK complexes in G1 phosphorylate the retinoblastoma family of proteins (pRb, p130, and p107) allowing the release of E2F transcription factors and upregulation of cellular genes to positively reinforce progression through this phase of the cell cycle (Massague, 2004).

Enzymatic activity of the cyclin-CDK complexes is controlled by availability of the cyclin subunits and phosphorylation status of the CDK subunits. While CDK levels remain relatively constant throughout the cell cycle, cyclin levels fluctuate as a result of transcriptional regulation and protein degradation (Sherr and Roberts, 1999). In the absence of mitogenic signals, cells must maintain CDKs in an inactive form to prevent cellular proliferation in adverse conditions. Such negative control is achieved by expressing CDK inhibitors (CKI). The CKI family of proteins includes p15, p16, p21, p27, and p57 (Sherr and Roberts, 1999). CKIs are some of the most frequently mutated genes in human cancers, and are also targets of transforming viruses. Viral oncoproteins, such as Tax, HBx and E6/E7, have evolved mechanisms to manipulate these regulatory pathways to promote cell cycle progression, and thus viral replication, in the absence of environmental stimuli.

Inhibition of CKI activity

The HTLV-1 oncoprotein Tax inhibits several CKIs through both direct interaction and transcriptional regulation. Tax directly binds to and inhibits p15 and p16, while it represses the transcription of p18 and p19 (Low et al., 1997; Suzuki et al., 1999; Li et al., 2003). In contrast, Tax activates the transcription of p21. Although this appears counterintuitive to the general role of CKIs in cell cycle progression, p21 is a unique CKI that can interact with multiple cyclin-CDK complexes to either inhibit or promote their kinase activity based on

stoichiometric levels of p21. For example, the binding of p21 to cyclin E-CDK2 represses activity of the complex, while the binding of p21 to cyclin D-CDK4 complexes promotes activity of the complex (Sherr and Roberts, 1999). The ability of Tax to increase p21 expression may reflect a requirement of the virus to drive the cell through G1 by enhancing cyclin D-CDK4 activity.

The HPV oncoproteins E6 and E7 also inhibit CKIs through direct protein interactions and transcriptional regulation. E6 represses p21 transcription while E7 binds and inactivates p21 and p27, subsequently enhancing the activities of both cyclin A-CDK2 and cyclin E-CDK2 complexes (Xiong et al., 1996; Funk et al., 1997; Jones et al., 1997). Similar to E6, the HBV transforming protein HBx also transcriptionally represses p21 and recent evidence indicates that it also has a negative effect on p27 (Kwun and Jang, 2004; Mukherji et al., 2007). Mukherji et al. (2007) reported decreased p27 associated with cyclin E-CDK2 complexes in HBx expressing cells due to rapid destabilization of p27. Although p16 transcription is repressed via enhanced p16 promoter methylation in HBV infected cells, there is currently no evidence that this is a specific effect of HBx (Zhu et al., 2007).

Enhancing cyclin-CDK complex activity

In addition to inhibiting CKIs, these three oncogenic viruses also influence the association and function of cyclin-CDK complexes. HTLV-1 Tax can interact with CDK4 and facilitate its binding to cyclin D2 leading to enhanced kinase activity, enhanced phosphorylation and proteasomal degradation of pRb, and early E2F release (Haller et al., 2002; Li et al., 2003). HBx expressing cells also demonstrate accelerated G1 to S phase transition resulting from early activation of CDK2. HBx directly binds to cyclin E and cyclin A resulting in increased interaction with CDK2 through an unknown mechanism (Mukherji et al., 2007). The enhanced rate and activity of CDK2 brought about by interacting with cyclin E and cyclin A promotes the emergence of cells from G1 and their entry into S phase (Benn and Schneider, 1995). Similar to HBx, HPV E7 complexes with cyclin E, however E7 and cyclin E do not directly interact. The E7 and cyclin E interaction requires the presence of the pRb related protein p107 with the resulting kinase active cyclin-CDK2 complex leading to phosphorylation of p107 (McIntyre et al., 1996). E7 can also activate the transcription of both cyclins E and A, thus increasing the probability of cyclin-CDK complex formation and activity (Schulze et al., 1998; Zariwala et al., 1998).

Phosphorylation of pRb

A third level of oncoviral effects on cell cycle progression occurs by inhibiting the growth suppressive activity of pRb. While HBx does not transcriptionally affect or directly bind pRb, its ability to activate upstream cyclin-CDK complexes leads to hyper-phosphorylated pRb and increased free E2F. HTLV-1 Tax, however, can directly interact with hypo-phosphorylated pRb resulting in premature proteasomal degradation of pRb (Kehn et al., 2005). HPV E7 can also bind pRb and the related proteins p107 and p130 to decrease their half-lives and limit their concentrations, which ultimately results in increased free E2F in the absence of a cellular stimulus (Boyer et al., 1996). The combined effects of these viruses on CKI inhibition, enhanced cyclin-CDK activity and increased pRb phosphorylation results in increased cell cycle progression. The more rapid onset of S-phase, resulting in increased

proliferative potential and deregulation of cell cycle progression by these viral oncoproteins, is one mechanism to accomplish the viral imperative to replicate.

Dysregulation of Cell Cycle Checkpoint Controls

In addition to monitoring and responding to environmental stimuli, cells must monitor the integrity of their DNA to ensure that deleterious mutations are not incorporated into the genome. Proliferating cells can conditionally stop cell cycle progression in response to genomic injuries and DNA replication errors by inducing the DNA damage checkpoint response, which allows time to repair DNA lesions. This pathway is initiated by activating the DNA damage sensors ATM and ATR, two related protein kinases that phosphorylate a large number of protein substrates involved in cell cycle control and DNA repair (Bartek and Lukas, 2001). The substrates of these kinases include the tumor suppressor protein p53 and checkpoint kinases 1 and 2 (Chk1 and Chk2). Phosphorylation of p53 by activated ATM/ATR increases its stability and function as a transactivator. During G1/S transition, p53 activates transcription of the CKI p21 which, in turn, binds to and inhibits CDK2, causing cell cycle arrest while the cell attempts to repair the DNA damage. Anti-growth signals such as checkpoint activation can limit the replication of oncogenic viruses, particularly if the checkpoint is activated in response to viral infection. These three viruses have therefore evolved mechanisms to inhibit the function of proteins such as the checkpoint kinases and p53, major mediators of cell cycle arrest, DNA repair and apoptosis.

Altering p53 function

p53, the “guardian of the genome,” is mutated in about 60% of human cancers and is a major target of viral oncoproteins. Activation of this tumor suppressor leads to a delay in the cell cycle to allow time to repair damaged DNA. p53 is also directly involved in some DNA repair processes. If the DNA damage is too extensive to be fixed, p53 mediates the initiation of apoptosis. Thus, defects in p53 function can adversely affect multiple pathways making p53 a major target of viral oncoproteins. The HPV oncoprotein E6 directly interacts with p53 to promote p53 ubiquitination via a proteasome-dependent pathway (Scheffner et al., 1990). As a result, cells expressing E6 cannot accumulate high levels of p53 in response to DNA damage and thus, cannot activate damage-induced checkpoints. HBx also directly interacts with p53 but rather than being degraded, HBx prevents p53 from interacting with DNA, thereby causing transcriptional downregulation of p53 target genes, including p21 (Wang et al., 1994). Unlike E6 and HBx, Tax does not interact with p53. Rather, Tax inhibits p53 function by alternate mechanisms including constitutive p53 phosphorylation (Pise-Masison et al., 1998), sequestration of the transcriptional co-activators p300/CBP (Tabakin-Fix et al., 2006), and NF- κ B-dependent sequestration of phosphorylated p53 (Pise-Masison et al., 2000). Collectively, the effects of Tax on p53 deplete HTLV-1 infected cells of functionally active p53 protein. Since p53 is crucial to the DNA damage response, its inhibition by viral oncoproteins leads to cell cycle progression and DNA replication in the presence of damage, resulting in the incorporation of mutations into the genome.

Bypassing the cell cycle checkpoints

The effects of viral oncoproteins on p53 reach beyond the G1/S checkpoint. E6 has been shown to abrogate the G2/M and mitotic spindle checkpoints in a p53 dependent manner (Thompson et al., 1997). Additionally, deregulation of p53 by E6/E7 has been shown to correlate with the upregulation of Plk-1, Aurora-A and cdk1 (Patel et al., 2004), kinases which play essential roles in the spindle checkpoint. Overexpression of these kinases correlates with increased centrosome amplification and chromosomal instability (Meraldi et al., 2002).

While these and other studies provide insight into how these viruses promote cell cycle progression, there has been little focus on the upstream checkpoint response. Recent work on Tax has shown that it can also bind to the DNA damage transducers Chk1 and Chk2, and inhibit their functions (Park et al., 2004, 2006). Chk1 and Chk2 are phosphorylated and activated by the ATR and ATM kinases, respectively. Activated Chk1 and Chk2 subsequently target downstream proteins to promote G1/S or G2/M arrest. The interaction of Tax with Chk1 was found to abolish Chk1 kinase activity and inhibit G2 arrest in response to γ -irradiation (Park et al., 2004). The interaction of Tax with Chk2 also decreased Chk2 kinase activity and attenuated apoptosis in response to γ -irradiation (Park et al., 2006). Recent studies have shown that Tax can prevent the egress of Chk2 from chromatin, where it is normally phosphorylated by ATM/ATR, thus preventing Chk2 from phosphorylating downstream proteins, such as p53 (Gupta et al., 2007). The effects of Tax on Chk1 and Chk2 provide new insight into the mechanisms used by oncogenic viruses to bypass both the DNA damage response and cell cycle checkpoints.

Inhibition of Cellular DNA Repair

Temporary inhibition of the cell cycle is only one arm of the checkpoint response. The cell must also repair damaged DNA before resuming the cell cycle. The mammalian genome is exposed to a plethora of stresses ranging from endogenous insults to chemical mutagens and cells have evolved mechanisms to respond to specific forms of damage. These repair pathways include base excision repair (BER) and nucleotide excision repair (NER) to remove bulky lesions formed from exposure to chemicals or UV-irradiation, and homologous recombination (HR) and non-homologous end joining (NHEJ) to repair double stranded breaks (Sancar et al., 2004). While HTLV-1, HBV and HPV do not directly induce DNA lesions, they have been shown to affect cellular repair mechanisms resulting in an accumulation of mutations in the host cell genome.

Base excision repair

BER is initiated by a glycosylase that recognizes minor DNA backbone distortions resulting from events such as mismatched bases. The specific glycosylase “flips” out the aberrant nucleotides, thereby recruiting an endonuclease and DNA polymerase β (pol β), coupled with the XRCC1 ligation complex, to the site to excise and fill in the nucleotide gap (Sancar et al., 2004). There is no current evidence that HBx inhibits BER, however both Tax and E6 affect the function pol β , which is essential for BER. Tax represses transcription of pol β (Jeang et al., 1990) thus depleting enzyme availability, while E6 interacts with XRCC1 to

alter pol β function, possibly by displacing the polymerase from the repair complex (Iftner et al., 2002).

Nucleotide excision repair

Removal of bulky DNA lesions requires the NER complex comprised of RPA, XPA, XPB, XPC, XPD, TFIIH, XPG, XPF-ERCC1, UV-DDB and PCNA proteins. RPA, XPA and the XPC-TFIIH complex independently assemble at sites of damage. The XPC-TFIIH complex includes two helicases, XPB and XPD, which unwind the damaged DNA allowing an incision complex to remove the lesion. Filling in of the gap is then accomplished by DNA polymerases δ/ϵ with the aid of the trimeric sliding clamp, PCNA which increases the processivity of the error free DNA polymerase δ (Sancar et al., 2004). Tax has been shown to suppress NER through transcriptional upregulation and activation of PCNA (Ressler et al., 1997), which increases the processivity of the error prone DNA polymerase δ (Kao and Marriott, 1999; Kao et al., 2000; Lemoine et al., 2000). By increasing PCNA expression, Tax presumably increases the frequency of mutations incorporated into the cellular genome, by promoting the use of error prone DNA polymerases during NER. HBx, on the other hand, affects the function of XPB and XPD helicases by preventing their interaction with p53 (Prost et al., 1998; Jia et al., 1999). HBx can also directly interact with both XPB and XPD proteins to enhance helicase activity of the TFIIH complex. A third level of HBx effect on NER involves its interaction with the UV-DDB complex. This complex is involved in recognizing DNA damage much like the XP proteins. HBx has been shown to bind and inhibit this complex, thereby diminishing NER activity (Lee et al., 1995). The HPV E6 protein also interferes with NER, but uses a different mechanism than Tax or HBx. E6 degrades O6-methylguanine-DNA methyltransferase (MGMT), an enzyme that functions to correct DNA lesions produced by endogenous mutagens and alkylating agents (Srivenugopal and Ali-Osman, 2002).

Double strand break repair

While viral oncogenes exhibit multiple effects on single strand DNA repair pathways, their effects on double strand break repair is not as extensively studied. Double strand breaks can be repaired by either HR or NHEJ. Currently, there is no evidence to suggest that oncogenic viruses affect the HR pathway, but there is emerging evidence that these viruses may affect NHEJ. In NHEJ, double strand breaks are bound by the Ku70/Ku80 heterodimer. These proteins recruit DNA-PKcs and the ligase 4-XRCC4 complex to ligate the two broken ends (Sancar et al., 2004). While HBx has not been linked to double strand break repair, HPV E6 and HTLV-1 Tax have recently been shown to disrupt NHEJ. Cells expressing E6 exhibit decreased DNA end joining repair in a p53-dependent manner (Shin et al., 2006). Using both in vivo and in vitro models, Shin et al. demonstrated that expression of E6 leads to a decrease in error-free DNA end-joining repair and a more dramatic increase in error-prone NHEJ. Furthermore, they determined that these effects depend upon the interaction of E6 with p53, which inhibits the post-translational modification and stabilization of p53.

Preliminary evidence also suggests that Tax can interfere with NHEJ. A study in Chinese Hamster Ovary (CHO) cells examined the effects of Tax on the formation of micronuclei (MN) as a measure of cytogenetic damage in cells. Tax transfected CHO cells exhibited

significantly more MN than untransfected CHO cells, suggesting an increased presence of free double-stranded DNA ends in the Tax-expressing cells. While a precise mechanism for this phenomenon has not been described, it is interesting to note that HTLV-1 transformed cells have reduced levels of Ku80 protein. Ku80 and Ku70 proteins are known to bind free DNA ends and facilitate DNA end joining. The fact that Ku80 null cells also display increased MN suggests that Tax may also inhibit DNA end joining (Majone et al., 2005).

The question of why viruses might have evolved mechanisms to inhibit DNA repair often arises since maintaining DNA integrity would benefit both host cell survival and virus stability. It is possible that the proteins targeted by viral oncogenes have alternate, yet to be elucidated, functions that benefit the virus. For example, it has recently been found that the interaction of HBx with DDB1, a member of the UV-DDB complex, results in increased viral mRNA levels and is necessary for viral replication (Leupin et al., 2005). Thus the inhibitory effects of HBx on the NER pathway could be a byproduct of sequestering DDB1 to promote viral replication. It has also been suggested that DNA repair proteins may function like tumor suppressor proteins, since activation of DNA repair pathways may lead to cellular senescence or death of oncogene-transformed cells, resulting in delay or prevention of tumorigenesis (Bartek et al., 2007). Viral oncogenes may have evolved to suppress DNA repair pathways in order to dismantle this tumorigenic barrier, which is often activated in response to viral oncogene expression.

Promoting Cell Survival

A hallmark of cancer cells is the ability to evade apoptosis, or programmed cell death. This very tightly regulated pathway plays an important role in fetal development and is also the process by which cells that could be detrimental to the organism are eliminated, such as cells containing DNA damage or those that are virally infected. There are two major apoptotic signaling pathways, the first requires signaling through the TNF-receptor family and the other is mitochondria mediated.

Mitochondrial-dependent apoptosis results in cytochrome *c* release and activation of the apoptotic protease activating factor-1 (APAF-1). APAF-1 binds to procaspase-9 to form the mitochondrial 'apoptosome' which subsequently activates downstream caspases. Members of the Bcl-2 protein family, which are either translocated to, or located at the mitochondrial membrane, also play a major role in this pathway. These proteins modulate permeability of the mitochondrial membrane and thus the extent of cytochrome *c* release (Vermeulen et al., 2005).

The TNF or death receptor family includes Fas receptor and TNF-receptor 1 (TNF-R1). Binding of Fas ligand to the Fas receptor leads to receptor trimerization and recruitment of adaptor proteins including the Fas-associated death domain protein (FADD) to the cytoplasmic domain of the receptor. FADD subsequently recruits procaspase-8, which becomes enzymatically active when cleaved and can activate downstream effector caspases. TNF receptor 1 is activated in a similar manner to Fas. Binding of TNF- α to its receptor results in receptor trimerization and binding of the adaptor, TNF-R-associated death domain protein (TRADD), which interacts with FADD to aid in recruiting procaspase-8 (Vermeulen

et al., 2005). HTLV-1, HBV and HPV have all evolved mechanisms to bypass programmed cell death. Since apoptotic pathways can be induced by cytotoxic T lymphocytes in response to viral infection, it is imperative for these viruses to target these pathways as a means of survival. Here we will discuss the effects of these viruses on Fas and TNF-R1-mediated apoptosis.

Fas-mediated apoptosis

T-cells isolated from Tax transgenic mice have been shown to be resistant to Fas-mediated apoptosis (Kishi, 1997). Recent work analyzing the anti-apoptotic effects of Tax found that Tax increased the expression of c-FLIP (Okamoto et al., 2006), an anti-apoptotic protein that inhibits Fas-mediated apoptosis by directly interacting with FADD and suppressing procaspase-8 activation (Vermeulen et al., 2005). Furthermore, suppression of c-FLIP in these cells by shRNA knockdown sensitized the Tax-expressing cells to apoptosis (Okamoto et al., 2006). HPV E6 protein has also been shown to bind directly to FADD and inhibit Fas-mediated apoptosis (Filippova et al., 2004). Interaction with E6 results in ubiquitination and degradation of FADD, thus preventing signaling to downstream procaspases. Similar to Tax, hepatocytes from HBx transgenic mice are resistant to Fas-mediated apoptosis, however the mechanism of this programmed cell death evasion have not been extensively studied (Keasler et al., 2006). HBx has however been shown to inhibit caspase-3 and caspase-8 activity in a p53-independent manner, but further mechanistic details remain to be elucidated (Gottlob et al., 1998a; Diao et al., 2001).

TNF-R1 mediated apoptosis

In addition to inhibiting Fas-mediated apoptosis, both Tax and the HPV E6 oncoproteins also target the TNF-R1 pathway. Tax has been found to transcriptionally upregulate c-IAP2 in HTLV-1 transformed cells in an NF- κ B dependent manner (Waldele et al., 2006). The anti-apoptotic protein c-IAP2 binds caspases 3, 7, and 9 and inhibits their catalytic activities. E6 also inhibits TNF-mediated apoptosis by binding to the death domain of TNF-R1, thus inhibiting its interaction with TRADD (Filippova et al., 2002). HPV E6 also inhibits TNF-mediated apoptosis, but by a different mechanism. E6 directly binds to Bak, a pro-apoptotic protein that oligomerizes with Bax at the mitochondrial membrane in response to caspase-8 activation resulting in mitochondrial mediated cell death (Vermeulen et al., 2005). Similar to its effects on p53, E6 mediates ubiquitination and degradation of Bak via the ubiquitin-proteasome pathway (Thomas and Banks, 1998). The effects of E6 on apoptosis appear to be dose dependent as cells expressing high levels of E6 are more sensitive to apoptosis induced by TNF than cells expressing low levels of E6 (Filippova et al., 2005).

Conclusion

This review has focused on the molecular mechanisms of transformation associated with three human oncogenic viruses. While we have focused on specific aspects of viral transformation mechanisms, it should be noted that in some cases these viruses can exert dual effects on some of the pathways described. Although there is extensive information on the cellular effects of viral oncoproteins, we have tried to briefly compare and contrast the transforming properties of HTLV-1, HBV, and HPV. Similar to the mutational process of

cancer induction, these viruses affect cellular processes required for cell cycle progression, DNA damage response and cell death. We have highlighted some of the major effects that these viral oncogenes exert on these pathways in an effort to promote viral replication and survival. While the transcriptional and cell cycle effects of these viruses are well studied, there are still black holes in our understanding of how oncogenic viruses influence the cell. Of particular importance is the inhibition of DNA damage signaling pathways by these viral oncoproteins. With the recent suggestion that DNA repair pathways have a tumor suppressor effect in response to oncogene activation, and the fact that defects in these pathways quickly lead to the accumulation of mutations, it is of great interest to understand how viral oncoproteins influence cellular responses to environmental damage and cellular stress. Further research into cellular factors targeted by viral oncoproteins and their effects on viral replication could provide new insight into the multiple functions of cellular proteins and provide a better understanding of why viruses have evolved to target these cellular functions.

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