PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 9.

# Human tumour viruses

*Paul F. Lambert and Lawrence Banks*

Among the biological agents reviewed in Volume 100B of the *IARC Monographs* (IARC, 2012) are several oncogenic viruses that are strictly species-specific, causing cancer in humans only. For this reason, the question about tumour site concordance between humans and experimental animals is not easy to answer for these agents, because cancer bioassays in animals are often lacking, and hence a proper comparison between data in humans and in experimental animals is not obvious. In this chapter, some aspects of this issue are discussed.

The use of animals as surrogate hosts for the study of human tumour viruses is often problematic, because species specificity limits the feasibility of this approach for most of these viruses. One exception is human T-cell lymphotropic virus type 1 (HTLV-1): in addition to its ability to infect humans, this virus can infect several other species – including rabbits, rats, and monkeys – and it does induce adult T-cell leukaemia/lymphoma (ATLL), albeit in monkeys only.

For other human tumour viruses, the use of humanized severe combined immunodeficiency (SCID) mice, in which the human target cell for the virus is placed in a murine host, can provide a platform for in vivo infection. However, except for Epstein–Barr virus (EBV), which causes lymphoproliferative diseases in New World monkeys and in humanized SCID mice, the use of surrogate hosts has not proven very useful for defining tumour site concordance between humans and experimental animals.

Animal models for human tumour viruses that make use of animal viruses are scarce. In fact, although many animal viruses that infect non-human primate species are related to the human tumour viruses, the incidence of cancer is low in these species (as it is in humans), which renders cancer studies costly and difficult. Moreover, animal models for tumour viruses in non-primate species often do not accurately reflect the mechanism of

the disease caused by the cognate human tumour virus. For instance, woodchuck hepatitis virus induces hepatocellular carcinoma (HCC) that is histopathologically very similar to that caused by hepatitis B virus (HBV) in humans, but it does so through a different mechanism.

Transgenic mouse models provide a powerful tool in mechanistic studies on the role of individual viral genes in cancer. Indeed, for several of the human tumour viruses described in Volume 100B of the *IARC Monographs*, transgenic mouse studies provide important mechanistic evidence. However, such transgenic models are inadequate for understanding the cancer etiology in the context of natural viral infection.

For several of the human tumour viruses classified by IARC in Group 1 (*carcinogenic to humans*), a number of studies with surrogate hosts, with cognate animal viruses, and with transgenic mouse models are reviewed below.

## Epstein–Barr virus (EBV)

Human peripheral blood leukocytes injected into SCID mice increase in number and survive for at least 6 months. These mice secrete human immunoglobulin and show a specific human antibody response after immunization. The major cell populations present in peripheral blood leukocytes are found in the lymphoid tissue and blood of the SCID mice recipients, although relative proportions may differ. Mice injected with peripheral blood leukocytes from EBV-seropositive donors often develop EBV-positive B-cell lymphomas (Mosier et al., 1988).

In a later study, a highly immunodeficient mouse strain (NOG) was injected with haematopoietic stem cells from human cord blood. These mice are able to reconstitute most major components of the human haematolymphoid system including T cells, B cells, natural killer cells, macrophages, and dendritic cells, and this humanized mouse model can simulate key aspects of EBV infection. Inoculation with a high dose of EBV caused a B-cell lymphoproliferative disorder in these mice, with histopathological findings and latent EBV gene expression similar to those in immunocompromised patients. Inoculation with a low dose of EBV resulted in apparently asymptomatic persistent infection. The number of activated CD8-positive T cells increased considerably in the peripheral blood of the infected mice, and various assays demonstrated an EBV-specific T-cell response. In addition, immunoglobulin M (IgM) antibodies specific to the EBV-encoded protein BFRF3 were detected in serum from infected animals (Yajima et al., 2008).

Inoculation of cotton-top tamarins – a type of New World monkey – with EBV induced multiple EBV genome-positive malignant large-cell lymphomas that closely resembled the EBV genome-positive B-cell lymphomas observed in human allograft recipients (Young et al., 1989a). The tumours in tamarins expressed EBV nuclear antigen 1 (EBNA1) and EBNA2, EBNA leader protein, and the latent membrane protein (LMP). The expression of EBNA2 and LMP in these lymphomas in tamarins strengthens their resemblance to lymphomas observed in human transplant recipients, because these tumours in humans are also EBNA2-positive and LMP-positive (Young et al., 1989b). Both proteins are important effector molecules of EBV-induced B-cell transformation in vitro, and their expression in these lymphomas in monkeys and humans provides strong support for a direct oncogenic role of EBV in vivo.

LMP1 of EBV can transform rodent fibroblasts and is expressed in most of the human cancers associated with EBV infection. Three strains of *LMP1* transgenic mice were established that express LMP1 under the control of the immunoglobulin heavy chain promoter/enhancer. Mice of all three strains developed lymphoma, the incidence of which increased considerably with age: after 18 months, 42% of the transgenic mice had developed lymphoma. LMP1 was strongly expressed in the lymphoma tissues but was hardly expressed in normal lymphoid tissues. These results show that *LMP1*, without the expression of other EBV genes, is oncogenic in vivo, and indicate that the LMP1 protein is a major contributing factor to the development of EBV-associated lymphomas (Kulwichit et al., 1998).

The role of LMP1 was also studied in the epidermis of *LMP1* transgenic mice. Epidermal cells that carried the transgene showed a 2–3-fold increase in the mitotic index and an increased expression of proliferative cytokeratin markers. This is direct evidence that LMP1 induces proliferation in otherwise normal epithelial cells in vivo. When treated topically with 7,12-dimethylbenz[*a*]anthracene, *LMP1* transgenic mice developed small papillomas more rapidly and in larger numbers than did non-transgenic controls. Furthermore, LMP1 could replace 12-*O*-tetradecanoylphorbol-13-acetate in promoting tumour formation, but it inhibited expansion and did not stimulate progression of the papillomas to carcinomas, or to more malignant spindle cell carcinomas.

These data show that early in the carcinogenic process, LMP1 acts as a tumour promoter after chemical initiation, but it may also block expansion or progression of benign lesions (Curran et al., 2001).

A further study on *LMP1* transgenic mice showed that they have a higher incidence of lymphoma and that the progression to lymphoma correlates with higher expression levels of LMP1, compared with non-transgenic controls. Although LMP1 is expressed in all B lymphocytes of the transgenic mice, lymphoma develops in a specific subset, the B-1a lymphocytes, which is a population predisposed to clonal expansion with age. The malignant lymphocytes show constitutively active Stat3 signalling, have decreased levels of p27, and display activated Akt and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathways, properties that are associated with promoting growth and survival of B lymphocytes. The transgenic lymphomas mirror multiple aspects of EBV-induced tumours; this suggests that these properties of LMP1 are major factors in cancer development (Shair et al., 2007).

Transcripts of *LMP2A* can be expressed in resting virus-carrying B lymphocytes in healthy individuals – the reservoir of persistently latent EBV. In *LMP2A* transgenic mice, a block in surface immunoglobulin rearrangement results in the generation of B-cell receptor-negative cells, which normally would undergo apoptosis. *LMP2A* transgenic B cells develop and survive without a B-cell receptor. These data indicate that LMP2A imparts developmental and survival signals to B cells in vivo (Merchant et al., 2000). Furthermore, when co-expressed with human MYC, LMP2A accelerates the development of B-cell lymphomas in a transgenic mouse model (Bieging et al., 2009; Bultema et al., 2009). A more recent study has also shown that LMP1 and LMP2 can cooperate in the induction of epithelial squamous cell carcinomas (SCCs) when co-expressed under the control of a keratin 14 (K14) promoter in transgenic mouse models (Shair et al., 2012).

Establishment of long-term latent infections with EBV was possible in a humanized mouse model that was challenged either with wild-type EBV or with a replication-defective virus. B-cell lymphomas developed in both cases, but at a higher frequency after infection with the wild-type virus, indicating a potential role for lytic virus infection in the development of malignancy (Ma et al., 2011).

For EBV, the overall concordance between the animal models and humans with respect to the types of tumour and the identity and function of the major oncogenes and oncogene products is high.

## Hepatitis B virus (HBV)

Interactive viral–chemical hepatocarcinogenesis was studied in woodchucks (*Marmota monax*) inoculated as newborns with woodchuck hepatitis virus, which is closely related to the human HBV. Starting at age 12 months, the woodchucks were fed a diet containing aflatoxin $B_1$ ($AFB_1$) (at a high dose for 4 months and then at a lower dose for lifetime). Carriers of woodchuck hepatitis virus with or without treatment with the carcinogen $AFB_1$ developed a high incidence of pre-neoplastic hepatic foci, hepatocellular adenomas, and HCCs, but $AFB_1$ treatment resulted in a much earlier appearance of hepatic neoplasms and a higher incidence of HCCs. No hepatocellular adenomas or HCCs were seen in non-infected woodchucks that received $AFB_1$ (but pre-neoplastic hepatic foci were seen), and no pre-neoplastic or neoplastic lesions were found in untreated controls. These results provide strong evidence of a synergistic hepatocarcinogenic effect of viral infection and dietary $AFB_1$ intake (Bannasch et al., 1995).

Transgenic mice that contain the *BglIIA* fragment of HBV under the transcriptional control of the mouse albumin promoter overexpress the HBV large envelope polypeptide and accumulate toxic quantities of hepatitis B surface antigen (HBsAg) in their hepatocytes. As a result, the mice develop severe, prolonged hepatocellular injury associated with an inflammatory response, followed by chronic hepatocellular regeneration, transcriptional deregulation, dysplasia, aneuploidy, hepatocellular adenoma, and eventually HCC. The incidence of HCC depends on the frequency, severity, and age of onset of the liver cell injury, which itself depends on the intrahepatic concentration of HBsAg and is influenced by genetic background and sex. Thus, the excessive expression of a single structural viral gene is sufficient to cause malignant transformation in this model. Similar events may be responsible for the development of HCC in humans after HBV infection, irrespective of the mechanism or mechanisms involved in the initial induction of liver cell injury (Chisari et al., 1989).

Transgenic mice overexpressing the HBV large envelope polypeptide suffer from hepatic injury as a result of accumulation of HBsAg (see the previous study). When treated with the hepatocarcinogens $AFB_1$

or diethylnitrosamine, these mice showed more rapid and more extensive formation of nodules, proliferation of oval cells, and development of adenomas and primary HCCs than did non-exposed transgenic mice. This suggests that the chronic liver damage and repair caused by overexpression of the HBV large envelope polypeptide act synergistically with chemical hepatocarcinogens to produce liver neoplasia (Sell et al., 1991).

To explain the synergistic hepatocarcinogenic effect of viral infection and dietary $AFB_1$ intake, it was suggested that infection with HBV and associated liver injury might alter the expression of carcinogen-metabolizing enzymes. This hypothesis was tested in the HBV transgenic mouse model described in the previous study. In these mice, the expression levels of the cytochrome P450 isozymes Cyp2a5 and Cyp3a – both involved in $AFB_1$ metabolism – were examined. Increases in the expression of and alterations in the distribution of Cyp2a5 were age-dependent in these mice and were associated with the extent of liver injury. Cyp3a expression was also increased, but this was less clearly related to age. These data show that expression of specific cytochrome P450 isozymes is altered in association with overexpression of the HBV large envelope polypeptide and the ensuing liver injury in this mouse model. This may have general relevance to human HCC, the etiology of which is associated with a diverse range of liver-damaging agents (Kirby et al., 1994).

The HBV X protein (HBx) is highly multifunctional. In transgenic mouse models, the expression of HBx promotes HCC (Yu et al., 1999). More recent studies have also shown cooperation between HBx and K-*ras* mutation in the development of HCC in transgenic mice (e.g. Ye et al., 2014). A truncated form of HBx that is commonly found in human HCCs also exhibits tumour-forming activity in association with exposure to diethylnitrosamine in transgenic mouse models of HCC (Quetier et al., 2015).

For HBV, the overall concordance between the animal models and humans with respect to the types of tumour is high.

## Hepatitis C virus (HCV)

### *Role of the HCV core protein in HCV-induced steatosis*

Several histological features in the liver are characteristic of chronic hepatitis C: bile duct damage, lymphoid follicles, and steatosis (fatty change). It has been suggested that the core protein of HCV functions as a transcriptional regulator that induces phenotypic changes in hepatocytes. Two independent strains of transgenic mice carrying the HCV core gene developed progressive hepatic steatosis, confirming that the core protein plays a direct role in the development of steatosis, which characterizes hepatitis C. These mice express the core protein in the liver at concentrations similar to those in the liver of patients with chronic hepatitis C. This transgenic mouse system may be a good animal model for the study of pathogenesis in human HCV infection (Moriya et al., 1997).

In a further study by the same group, several parameters of oxidative stress and redox homeostasis were measured in these transgenic mice. At age 3–12 months, the mice showed similar concentrations of phosphatidylcholine hydroperoxides and phosphatidylethanolamine in liver tissue homogenates as the non-transgenic controls. In contrast, the level of phosphatidylcholine hydroperoxides was increased nearly 2-fold in transgenic mice after age 16 months. In addition, catalase activity was increased and the concentrations of total and reduced glutathione were decreased. These mice show steatosis without inflammation early in life, and finally develop HCC from age 16 months. The HCV core protein thus alters the oxidant–antioxidant status in the liver in the absence of inflammation and may thereby contribute to or facilitate the development of HCC after HCV infection (Moriya et al., 2001).

### *Transgenic mice carrying the complete HCV polyprotein*

In a study to determine whether expression of HCV proteins alters hepatic morphology or function in the absence of inflammation, transgenic C57BL/6 mice carrying the complete viral polyprotein (*FL-N* transgene) or viral structural proteins (*S-N* transgene) were compared with non-transgenic littermates for altered liver morphology and function. No inflammation was seen in the livers of transgenic mice, but mice expressing either transgene developed age-related hepatic steatosis. The numbers of apoptotic or proliferating hepatocytes were not increased significantly. Hepatocellular adenoma or HCC developed in older male mice expressing the *FL-N* or the *S-N* transgene, but the incidence was increased significantly only in *FL-N* transgenic mice. Neither of these tumours was observed in age-matched non-transgenic mice. Expression of viral proteins gave rise to common pathological features of hepatitis C in the absence of a specific antiviral immune response, which suggests a

metabolic or genetic host susceptibility for HCV-associated HCC (Lerat et al., 2002).

In a subsequent study by the same group, the mechanisms underlying HCV-induced defects in lipid metabolism were studied in transgenic mice that expressed the full viral protein repertoire at levels corresponding to those seen in natural human HCV infection. Expression of the full-length HCV open reading frame was associated with hepatocellular steatosis, impaired triglyceride secretion, and nuclear translocation of sterol regulatory element-binding protein 1c (SREBP1c), followed by increased transcription of lipogenic enzymes. Stress markers in the endoplasmic reticulum were expressed at similar levels in HCV transgenic mice and in non-transgenic controls. Transgenic mice expressing the full-length HCV polyprotein have reduced plasma triglyceride concentrations and develop hepatocellular steatosis in the same way as do patients with HCV infection (Lerat et al., 2009).

### *Effect of peroxisome proliferator-activated receptors*

Peroxisome proliferator-activated receptor alpha (PPARα) is a central regulator of triglyceride homeostasis and a mediator of hepatocarcinogenesis in rodents. In a study to determine the role of PPARα in HCV core protein-induced disease, double transgenic mice were generated that carried *Pparα* (homozygous, heterozygous, and null) and the HCV core protein gene (*HCVcp*) as transgenes. Severe steatosis was observed only in *Pparα*$^{+/+}$*:HCVcp* mice, as a result of higher fatty acid uptake and decreased mitochondrial β-oxidation due to breakdown of mitochondrial outer membranes. HCC developed in about 35% of *Pparα*$^{+/+}$*:HCVcp* mice by age 24 months, but no tumours were seen in the other genotypes. These phenomena were closely associated with sustained PPARα activation: in *Pparα*$^{+/-}$*:HCVcp* mice, PPARα activation and the related changes did not occur despite the presence of a functional *Pparα* allele. Persistent activation of PPARα is essential for the pathogenesis of hepatic steatosis and HCC induced by HCV infection (Tanaka et al., 2008).

### *Conclusions*

In the absence of animal models that develop HCC in the context of an HCV infection, various groups have reported the use of transgenic mouse models. Studies with mice expressing HCV replicons, polyproteins, or single HCV proteins as transgenes, alone or in combination, under the control of liver-specific promoters have been described by several groups (Dorner et al., 2011, 2013). There is good concordance between the outcomes observed in these mice and those in humans with HCV infection; however, questions remain about to what extent the results obtained with these experimental approaches reflect the pathological consequences of human HCV infection that contribute to HCC.

It remains a matter of considerable debate whether HCV causes HCC through a direct mechanism in which virally encoded genes contribute to carcinogenesis, or through an indirect mechanism, where the injury to the liver caused by HCV infection and host immune responses to that infection, such as inflammation, contribute to the onset of cancer. This issue may be resolved with the development of more physiological models that permit chronic and productive HCV replication. A recently developed model holds promise in this regard. Genetically engineered mice expressing two host restriction factors – human CD81 and occludin – can be infected with HCV, and in these mice sustained viraemia and infectivity can be observed for more than 12 months after infection, with the expected fibrotic and cirrhotic progression in the liver (Chen et al., 2014).

## Kaposi sarcoma-associated herpesvirus (KSHV)

### *Species specificity of KSHV*

After injection, KSHV can infect non-human primates (Renne et al., 2004), NOD/SCID mice (Parsons et al., 2006), and humanized SCID mice (Dittmer et al., 1999; Foreman et al., 2001; Wu et al., 2006; Wang et al., 2014). These infections do not result in the formation of tumours, but they confirm the viral tropism (with KSHV targeting B cells and endothelial cells) and drug susceptibility (to ganciclovir) in vivo.

In one report of KSHV infection in marmosets (*Callithrix jacchus*), viral replication was apparent in peripheral blood mononuclear cells, and a Kaposi sarcoma-like lesion developed on one of the animals (Chang et al., 2009). Viruses that are homologous to KSHV exist in the bank vole (*Myodes glareolus*), as Murid herpesvirus 68 (MHV-68), and in virtually all non-human primates (Fleckenstein and Ensser, 2007). The infection of macaques (*Macaca mulatta*) with rhesus rhadinovirus in the context of simian immunodeficiency virus (SIV) induces B-cell lymphoma and endothelial cell

hyperplasia (Mansfield et al., 1999; Wong et al., 1999). Several tumour graft models of Kaposi sarcoma and primary effusion lymphoma have been established (Boshoff et al., 1998; Staudt et al., 2004; Wu et al., 2005; An et al., 2006; Mutlu et al., 2007; Sin et al., 2007).

In humans, KSHV is associated with Kaposi sarcoma and primary effusion lymphoma. The transforming capacity of several individual KSHV proteins with respect to these two tumour types has been studied in experimental systems, particularly in transgenic mice.

## Studies in transgenic mice

Transgenic mice in which individual KSHV proteins are expressed are often used to replicate aspects of the pathogenesis of KSHV. However, single transgenic models have some limitations. Whereas lymphoproliferative lesions and lymphomas in mice are easily classified on the basis of histology and marker gene expression, this is more difficult for endothelial cell tumours, which are often referred to as Kaposi sarcoma-like lesions but can easily be mistaken for fibrosarcomas.

### *LANA*

The KSHV latency-associated nuclear antigen (LANA) is consistently expressed in all KSHV-associated tumour cells and was shown to bind the tumour suppressor proteins p53 and pRb. The contribution of this antigen to lymphomagenesis in vivo was investigated in transgenic mice that expressed LANA under the control of its own B-cell-specific promoter. All of the mice developed splenic follicular hyperplasia due to an expansion of IgM-positive, IgD-positive B cells, and 11% developed different types of lymphoma, among which were plasmacytoma, follicular B-cell lymphoma, small lymphocytic lymphoma, and composite lymphoma. These results imply that LANA can activate B cells and trigger the first step towards lymphomagenesis (Fakhari et al., 2006). The authors of the study speculated that in asymptomatic human carriers, infected B cells remain organ-resident and sustain latent persistence of KSHV; this would be consistent with the low frequency of KSHV genome-positive cells in peripheral blood lymphocytes of LANA-seropositive individuals (Whitby et al., 1995).

### *vFLIP*

The KSHV-encoded viral Fas-associated death domain-like IL-1-converting enzyme inhibitory protein (vFLIP) is expressed in latently infected cells and plays an important role in survival and proliferation of primary effusion lymphoma cells. One function ascribed to this protein is activation of NF-κB. Transgenic mice expressing vFLIP display constitutive activation of NF-κB pathways, show an enhanced response to mitogenic stimuli, and have an increased incidence of lymphoma. These results demonstrate that the KSHV-encoded vFLIP is an oncoprotein that could contribute to the development of lymphoproliferative disorders via constitutive NF-κB activation (Chugh et al., 2005).

### *K1 protein*

The K1 protein of KSHV is a transmembrane signalling protein. In transgenic mice that express the KSHV *K1* gene under the control of the simian virus 40 (SV40) promoter, tumours were observed that showed features of spindle cell (sarcomatoid) cancers and malignant plasmablastic lymphoma. The enhanced NF-κB activity in non-malignant lymphocytes of *K1* transgenic mice and the persistence of this activity in the lymphoma tumours that these mice develop suggest that the KSHV *K1* transgenic mouse may be a model of premalignancy (Prakash et al., 2002).

### *K cyclin*

KSHV encodes a cyclin D homologue, K cyclin, which is thought to promote viral oncogenesis. In cultured cells, expression of K cyclin not only triggers cell-cycle progression but also engages the p53 tumour suppressor pathway, which probably restricts the oncogenic potential of K cyclin (Verschuren et al., 2002). The tumorigenic properties of K cyclin were assessed in transgenic mice in which expression of K cyclin was targeted to B and T lymphocytes via the Eμ promoter/enhancer. About 17% of K cyclin transgenic mice had developed lymphoma by age 9 months, and all lymphomas had lost p53 activity. The critical role of p53 in suppressing K cyclin-induced lymphomagenesis was confirmed by the greatly accelerated onset of B and T lymphomagenesis in all K cyclin/$p53^{-/-}$ mice compared with K cyclin/$p53^{+/-}$ and K cyclin/$p53^{+/+}$ mice, but suppression of apoptosis does not appear to be the underlying mechanism, given the very high numbers of apoptotic cells observed in all Eμ-K cyclin/$p53^{-/-}$ thymic lymphomas (Verschuren et al., 2004).

### *vGPCR*

In transgenic mice, the expression of viral G protein-coupled receptor (vGPCR) by cells of endothelial origin triggers the development of an angioproliferative disease that resembles Kaposi sarcoma. It includes expression of angiogenic factors such

as placental growth factor, platelet-derived growth factor B, and inducible nitric oxide synthase by the vGPCR-expressing cells. Finally, continued vGPCR expression is essential for progression of the Kaposi sarcoma-like phenotype, and downregulation of vGPCR expression results in reduced expression of angiogenic factors and regression of the lesions. These findings implicate vGPCR as a key element in the pathogenesis of Kaposi sarcoma (Jensen et al., 2005).

Further support for a role of vGPCR in tumorigenesis has come from studies with MHV-68. In mice, this virus can replicate transiently before entering a latent state, but no lymphoproliferative disorders arise in immunocompetent animals. However, when a recombinant MHV-68 in which the vGPCR is replaced with the KSHV-derived vGPCR was used to infect mice, angiogenic lesions formed that had features characteristic of those seen in human Kaposi sarcoma lesions. The difference in activity between the wild-type MHV-68 and the recombinant MHV-68 was linked to differences in activation: the KSHV-derived vGPCR was constitutively active, whereas the murine vGPCR in MHV-68 was not (Zhang et al., 2015). This study provides compelling evidence for a direct role of vGPCR in the development of Kaposi sarcoma in a non-transgenic animal model.

For KSHV, the overall concordance between the animal models and humans with respect to the types of tumour and the identity and function of the major oncogenes and oncogene products is high.

## Human papillomaviruses

The cell-transforming capacity of human papillomavirus (HPV)-encoded proteins has been demonstrated in various cell lines. In particular, HPV type 16 (HPV16) had transforming potential in established rodent cells (Yasumoto et al., 1986), with the principal activity residing in the E7 oncoprotein (Vousden et al., 1988). The E5 and E6 proteins of both HPV16 and HPV18 also showed transforming potential in such assays (Pim et al., 1992). The E6 protein can inactivate p53 (Scheffner et al., 1990), and as a result, E6 can abrogate cell-cycle arrest induced by a variety of DNA-damaging agents, such as actinomycin D. The normal response to DNA damage, i.e. inhibition of DNA synthesis and increase in p53 protein levels, did not occur after treatment with actinomycin D of keratinocytes that had been immortalized with HPV16 E6/E7 (Kessis et al., 1993).

### *Transgenic models for HPV-associated cancers*

The first germline transgenic mouse model for HPV-associated cervical cancer was developed with a K14-HPV16 construct that contained the early genes of the virus under the control of the K14 transcriptional promoter, which directs the expression of these genes to the stratified epithelium of the oral cavity and the lower female reproductive tract. These mice did not develop cervical cancers spontaneously, but treatment with estrogen, sufficient to induce continuous estrus, led to a highly penetrant cervical cancer phenotype in the context of a progressive disease much like that seen in women, given that it was preceded by the onset of cervical intraepithelial neoplasia (CIN) of grades 1–3. As in women, the cancers preferentially arose in the transformation zone (Arbeit et al., 1994, 1996). All *K14-E7* transgenic mice treated with estrogen for 6 months developed high-grade dysplasia and/or cervical cancer, but *K14-E6* mice only developed cervical cancer after treatment with estrogen for 9 months (Riley et al., 2003; Shai et al., 2007, 2008).

An additional activity of HPV16 E7 that contributes to its oncogenicity is the ability to inactivate p21 (Shin et al., 2009). In transgenic mice carrying a tetracycline-regulated HPV16 *E7* transgene, the continued expression of E7 was found to be critical for the maintenance not only of cervical cancer but also of the dysplastic neoplasia that is recognized as its precursor lesion (Jabbar et al., 2009). This dependence on continued expression of E7 was observed even in the context of constitutive expression of HPV16 E6 (Jabbar et al., 2012).

Like E7, the E6 protein of HPV appears to contribute to cervical carcinogenesis through multiple activities. This protein is known to degrade p53 and other cellular targets through its interaction with the ubiquitin ligase E6AP. This enzyme was found to be critical for E6-mediated oncogenesis in the cervix (Shai et al., 2010). E6 is known to bind to several cellular proteins that contain PDZ domains (common structural regions of 80–90 amino acids in proteins involved in signalling). Transgenic mice expressing a mutant form of HPV16 E6 that is unable to bind to PDZ-domain proteins (Nguyen et al., 2003) had a reduced susceptibility to cervical cancer compared with mice expressing the wild-type E6 protein (Shai et al., 2007). It remains unclear which of the interactions with PDZ-domain proteins contribute to E6-mediated carcinogenesis in vivo, and how E7 is involved in this process (Simonson et al., 2005; Shai et al., 2007).

The same high-risk HPV types associated with cervical cancer are also linked to the development of anal cancer. In the same K14-HPV16 transgenic mouse models, the expression of the *E6/E7* transgenes gave rise to anal cancers in mice treated with 7,12-dimethylbenz[*a*] anthracene (Stelzer et al., 2010). As with the cervical lesions, the E7 oncoprotein also plays a dominant role in this case (Thomas et al., 2011).

### Role of estrogen in HPV-mediated cervical carcinogenesis

Estrogen is an important cofactor in the development of cervical cancers in HPV transgenic mouse models (Arbeit et al., 1996). Tumours arising in HPV16 transgenic mice – carrying *E7* or *E6/E7* as transgenes – treated with estrogen for 9 months were much larger than those observed after 6 months of treatment. When these mice were treated with estrogen for 6 months and then kept without treatment for 3 months, they had significantly fewer, smaller, and less aggressive tumours at 9 months than those seen in mice treated for the full 9 months; thus, tumour regression was seen in the 3-month period after treatment. Estrogen therefore plays a critical role not only in the genesis of cervical cancer but also in its persistence and continued development (Brake and Lambert, 2005).

In a later study, estrogen receptor alpha (ERα) was found to be necessary for development of cervical carcinogenesis in *K14-E7* transgenic mice: exogenous estrogen failed to promote either dysplasia or cervical cancer in *K14-E7/ERα*$^{-/-}$ mice (Chung et al., 2008). Interestingly, expression of ERα in the cervical stroma was required for cervical carcinogenesis in HPV transgenic mice (Chung et al., 2013); evidence for a role of stromal ERα has also been obtained in the context of human cervical cancer (den Boon et al., 2015). ERα antagonists were effective in eliminating cervical cancer and the precancerous lesions in this animal model (Chung and Lambert, 2009). The cervical cancers in these mouse models are strictly associated with atypical squamous metaplasia, which is believed to be the precursor of cervical cancer in women.

### High-risk HPV types and cancers of the head and neck

The same high-risk HPV types that are etiologically associated with anogenital cancers, particularly HPV16, are also associated with a subset of human head and neck squamous cell carcinomas (HNSCCs), most notably of the oropharynx (e.g. tonsils), the base of the tongue, and the upper oesophagus. The role of the HPV16 proteins E6 and E7 in HNSCC has been evaluated in HPV transgenic mice that express the two oncogenes *E6* and *E7* in the relevant tissues. These mice do not spontaneously develop HNSCC, but when treated with the synthetic carcinogen 4-nitroquinoline-*N*-oxide, they become more susceptible to head and neck cancers (Strati et al., 2006). The progressive disease observed in the mice treated with 4-nitroquinoline-*N*-oxide was similar to that seen in humans, and the cancers that occurred were primarily high-grade HNSCC, as observed in HPV-positive HNSCCs in humans. As with cervical cancer, *E7* proved to be the more potent oncogene (Jabbar et al., 2010), and the inactivation of pRb could not fully account for the role of the E7 protein (Strati and Lambert, 2007), whereas inactivation of both pRb and p107 could fully recapitulate the oncogenicity of E7 in HNSCC (Shin et al., 2012).

### Carcinogenic potential of epidermodysplasia verruciformis-associated beta HPV types in the skin

HPV types of the genus beta, specifically HPV8 and HPV38, are associated with a rare familial benign disease termed epidermodysplasia verruciformis. Patients with this disease are at an increased risk of SCCs of the skin at sun-exposed areas. K14-HPV8 transgenic mice expressing the early genes of HPV8 in the epidermis were susceptible to spontaneous development of both benign and malignant skin cancers (Schaper et al., 2005). Unlike what is seen for the mucosal HPV types, the viral E2 protein seems to play a major role in these HPV8-induced cancers, because expression of E2 alone also results in the development of skin cancer, a process that is accelerated after irradiation with ultraviolet (UV) light (Pfefferle et al., 2008).

In the case of HPV38, *K10-E6/E7* transgenic mice were highly susceptible to multistage skin carcinogenesis, specifically when treated with UVB radiation or chemical carcinogens (Dong et al., 2005). The synergy between cutaneous HPV types and UV radiation in the development of SCCs of the skin has also been studied in transgenic SKH-hr1 hairless mice expressing in their epidermis the *E6* and *E7* genes of HPV20, which is commonly associated with SCC observed in renal transplant recipients, or of HPV27, which is only associated with benign papillomas. Upon UV irradiation, both HPV20 *E6/E7* and HPV27 *E6/E7* transgenic mice were more

susceptible to tumours compared with non-transgenic mice, and the HPV20 *E6/E7* transgenic mice had an increased incidence of malignant tumours. Alterations in the expression of both p53 and p63 were noted in the transgenic mice exposed to UV radiation (Michel et al., 2006).

For HPV, the overall concordance between the animal models and humans with respect to the types of tumour caused by mucosal HPV types and the identity and function of the major oncogenes and oncogene products is high. There are important context-dependent differences in the function of the E6 oncoprotein, depending on the anatomical site.

## Human immunodeficiency virus type 1 (HIV-1)

Infectious agents can act as indirect carcinogens by causing immunosuppression. This has been shown for infection with HIV-1, which strongly increases the incidence of several human cancers. Strikingly, the majority of cancers associated with HIV-1 have another known infectious etiology, and HIV-1 infection increases their incidence considerably. Among these cancers, those associated with the herpesviruses KSHV and EBV are most strongly enhanced by immunosuppression. The same cancers are also enhanced by iatrogenic immunosuppression, as shown by their increased incidence in transplant recipients, which lends additional support to the notion that HIV-1 acts as a carcinogen mainly through this indirect effect. The most common cancers in individuals with HIV-1 infection are Kaposi sarcoma (caused by KSHV), lymphomas (many of which are EBV-positive), and cervical and anogenital carcinomas associated with HPV infection.

Because HIV-1 is species-specific, like the oncogenic herpesviruses EBV and KSHV, there are no ideal animal models for HIV-1-associated cancers. In contrast to what is observed with infectious agents that are directly oncogenic, the HIV-1 genome is not present in cancer cells. Therefore, any interaction between virus and host is indirect. Although none of the HIV-1-encoded proteins has been unequivocally shown to be directly oncogenic, some are associated with immunodeficiency, thereby indirectly promoting cancer development. In addition, there is evidence that some of the HIV-1-encoded proteins may promote cancer by other indirect mechanisms that are not dependent on immunodeficiency. There are also reports of transgenic mouse models containing HIV proviral transgenes. In some cases these animals develop lymphoproliferative disorders, including B-cell lymphomas, which show characteristics similar to those of B-cell lymphomas arising in patients with HIV infection (Curreli et al., 2013). Virus-encoded proteins including Nef, gp120, p17, and Tat have all been implicated in promoting B-cell hyperproliferation, although the strongest association in animals comes from work on the viral Tat protein.

### *Tat protein*

The multifunctional Tat protein is the only HIV-1 protein for which there is experimental evidence of a potential role in Kaposi sarcoma. Tat is an important regulator of viral transcription; it recruits cellular transcription factors to the HIV-1 promoter, strongly stimulates HIV-1 DNA transcription, and interacts with protein–kinase complexes (Cdk9/cyclin T1, Cdk2/cyclin E), protein phosphatases, and multiple other cellular proteins (Gatignol, 2007). Tat also affects the course of HIV-1-associated disease indirectly, because it is secreted by infected cells and can enter non-infected cells (Gupta and Mitra, 2007).

Evidence that Tat is involved in oncogenesis includes its ability to induce apoptosis in neighbouring non-infected cells when secreted from infected cells, thereby increasing the susceptibility of bystander CD4-positive T cells to death induced by cross-linking (Alimonti et al., 2003). This may contribute to the massive depletion of CD4-positive T cells by apoptosis, leading to the severe immunodeficiency seen in the acquired immune deficiency syndrome (AIDS). Tat has also been shown to stimulate the growth of Kaposi sarcoma cells (Aoki and Tosato, 2007). However, when cells are removed from Kaposi sarcoma lesions and expanded in vitro, they lose the KSHV genome, and the question remains whether the “Kaposi sarcoma” cells lacking KSHV used in most of these studies represent a valid model for Kaposi sarcoma.

To investigate the role of Tat in carcinogenesis, several studies have been carried out in vivo, in transgenic mice. Transgenic mice carrying a recombinant DNA sequence containing the early region of the BK virus and the HIV-1 *Tat* gene developed skin leiomyosarcomas, squamous cell papillomas and carcinomas, adenocarcinomas of skin adnexal glands, and B-cell lymphomas. Although the incidence of HCC was low, most animals showed liver cell dysplasia of variable degree. These mice were also affected by skin lesions resembling the early stages of Kaposi sarcoma (see also Vogel et al., 1988). The *Tat* transgene was

detected intact in all the organs of the transgenic mice, and the Tat protein was expressed in essentially all tissues and organs of these animals. These BK virus/*Tat* transgenic mice may be useful in studies of the role of Tat in AIDS-associated malignancies and of the pathogenesis of Kaposi sarcoma (Corallini et al., 1993).

Because the Tat protein stimulates cell proliferation, inhibits apoptosis, displays angiogenic functions, and may be involved in the pathogenesis of Kaposi sarcoma and other tumours arising in patients with AIDS, the BK/*Tat* transgenic mice (see the previous study) may be predisposed to tumour formation. When *Tat* transgenic mice were treated with urethane, the incidence of lung tumours and lymphomas was not different between the transgenic mice and the controls, whereas the incidence of pre-neoplastic lesions and tumours in the liver was significantly higher in *Tat* transgenic mice than in control mice. This remarkable effect of urethane observed in the liver may be due to a Tat-induced predisposition, manifested as a liver cell dysplasia, spontaneously affecting most of the *Tat* transgenic mice. Liver cell dysplasia may exert a promoting effect by stimulating proliferation of cell clones initiated by the mutagenic effect of urethane. In addition, liver cell dysplasia may enhance the progression to malignancy of the pre-neoplastic lesions induced by urethane. This study suggests a role of Tat in the promotion and progression of carcinogen-initiated tumours in patients with HIV-1 infection (Altavilla et al., 2004).

For HIV-1, the overall concordance between the animal models and humans with respect to the types of tumour is low.

## Human T-cell lymphotropic virus type 1 (HTLV-1)

HTLV-1 naturally infects humans, but the virus can be inoculated into different animals, including rabbits, rats, mice, and New World monkeys, with various effects (Lairmore et al., 2005). In rabbits and rats, HTLV-1 infection is persistent but does not lead to definite diseases.

Different monkey species are naturally infected with STLV-1, the simian analogue of HTLV-1, and several cases of adult T-cell leukaemia/lymphoma (ATLL) have been described in African green monkeys (Tsujimoto et al., 1987; Akari et al., 1998). Experimental infection with HTLV-1 of squirrel monkeys (*Saimiri sciureus*) caused a strong reduction in the proliferation rate of the CD4-positive T-cell population in those infected animals that were affected by a pathology similar to ATLL in humans (Debacq et al., 2005). Co-infection of rhesus macaques (*Macaca mulatta*) with HTLV-1 and SIV type 1 (SIV-1) increased the number of multilobulated lymphocytes ("flower" cells) in the circulation; this cell type is also seen in patients with ATLL. SIV-1 may have the potential to upregulate HTLV-1 and enhance expression of disease (Traina-Dorge et al., 2007). So far, non-human primates represent the only suitable animal model to study human ATLL.

The pX region of HTLV-1 encodes the regulatory genes *Tax* and *Rex*, and several accessory genes. Tax, a 40-kD phosphoprotein, is found mainly in the nucleus but also in the cytoplasm (Meertens et al., 2004). Interaction of Tax with several host factors results in transactivation of some genes, transrepression of others, modulation of the cell cycle, and dysregulation of apoptosis (Matsuoka and Jeang, 2007). The transforming ability of Tax was demonstrated in the Rat-1 fibroblast cell line in vitro in a soft agar assay, and in vivo in nude mice (Tanaka et al., 1990). These findings clearly show that Tax is oncogenic.

Several studies with animals transgenic for Tax clearly demonstrated that Tax expression leads to the induction of tumours, confirming that Tax is oncogenic in vivo. In *Tax* transgenic animals, the Tax protein was shown to be oncogenic, with the tumour type depending on the promoter used in each study. Mice that expressed Tax under the control of the granzyme B promoter developed tumours of natural killer cells (Grossman et al., 1995), and mice that expressed Tax via the lck promoter developed a disease that resembles ATLL (Hasegawa et al., 2006). The major difference between most of these animal tumours and ATLL in humans is the fact that a subset of human ATLL cells do not express Tax.

More recently, different models of humanized mice have been used to assess the effects of infection with HTLV-1 (Villaudy et al., 2011; Tezuka et al., 2014). In these cases, the mice developed ATLL-like leukaemic symptoms, including splenomegaly and lymphoma.

Cytogenetic analysis of ATLL cells has shown a common breakpoint cluster region in chromosome 10p11.2. Further analyses have shown that the transcription factor 8 (TCF8) is frequently disrupted by several mechanisms, including epigenetic silencing. Suppressed expression of TCF8 is associated with resistance to transforming growth factor beta (TGF-β). Mice carrying a mutation in TCF8 frequently developed thymic T-cell lymphoma, indicating that *TCF8* is

a tumour suppressor gene (Hidaka et al., 2008). There have been only few reports of cellular oncogenes in ATLL cells. When complementary DNA expression libraries derived from leukaemic cells of patients with ATLL were screened for the potential to transform NIH 3T3 mouse fibroblasts, a novel transforming gene, *Tgat*, was identified. Expression of *Tgat* in NIH 3T3 cells resulted in cellular transformation, indicated by anchorage-independent growth in semi-solid medium, and tumour formation in nude mice (Yoshizuka et al., 2004).

For HTLV-1, the overall concordance between the animal models and humans with respect to the types of tumour and the identity and function of the major oncogenes and oncogene products is high.

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