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VIRIAL INFECTIONS: REPLICATION AND PATHOGENESIS MECHANISMS TO THERAPY

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Murine Models of Chronic Viral Infections and Associated Cancers

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Abstract—Viruses are now recognized as bona fide etiologic factors of human cancer. Carcinogenic viruses include Epstein– Barr virus (EBV), high-risk human papillomaviruses (HPVs), hepatitis B virus (HBV), hepatitis C virus (HCV), human T-cell leukemia virus type 1 (HTLV-1), human immunodeficiency virus type 1 (HIV-1, indirectly), and several candidate human cancer viruses. It is estimated that 15% of all human tumors worldwide are caused by viruses. Tumor viruses establish long-term persistent infections in humans, and cancer is an accidental side effect of viral replication strategies. Viruses are usually not complete carcinogens, supporting the concept that cancer results from the accumulation of multiple cooperating events, in which human cancer viruses display different, often opposing roles. The laboratory mouse *Mus musculus* is one of the best in vivo experimental systems for modeling human pathology, including viral infections and cancer. However, mice are unsusceptible to infection with the known carcinogenic viruses. Many murine models were developed to overcome this limitation and to address various aspects of virus-associated carcinogenesis, from tumors resulting from xenografts of human tissues and cells, including cancerous and virus infected, to genetically engineered mice susceptible to viral infections and associated cancer. The review considers the main existing models, analyzes their advantages and drawbacks, describes their applications, outlines the prospects of their further development.

Keywords: murine models, chronic viral infection, viral carcinogenesis, viral oncogenes, xenograft, hepatitis B virus, hepatitis C virus, human immunodeficiency virus type 1 (HIV-1), Epstein–Barr virus, human T-cell leukemia virus type 1

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INTRODUCTION

The laboratory mouse *Mus musculus* provides one of the best experimental in vivo systems to model human pathologies, including viral infections and cancers. Several advantages are characteristic of murine models: mice are small in size, have a short lifespan, are simple to rear, and are highly similar to humans both molecularly and physiologically; their immune system is well understood; and their genome is entirely sequenced, suggesting a possibility of wide genetic modification. Murine models proved useful for understanding the biological processes that accompany tumor growth and conducting preclinical studies of anticancer therapies [1]. However, mice cannot be infected with the viruses that cause chronic infections in humans and are associated with carcinogenesis, including the hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus type 1 (HIV-1), Epstein–Barr virus (EBV), human T-cell leukemia virus type 1 (HTLV-1), high-risk human papillomaviruses (HPVs), etc. Many murine models of virus-associated tumors were developed to address various aspects of virus-associated carcinogenesis, from xenograft models based on explants of human tissues, including tumors, to genetically modified mice that are susceptible to viral infections and virus-associated tumors. The review considers the principles of model design and describes several examples of using murine models to study chronic viral infections and virus-associated carcinogenesis and to develop methods of their therapy.

XENOGRAFT MODELS: MICE WITH XENOGRAFTED HUMAN CELLS

Patient-Derived Xenografts in Immunocompromised Mice

Human cells and tissues grafted to murine models perform the same functions as in the human body. These models are very useful for direct studies of human pathogens and mechanisms of infection.

To obtain a model of virus-associated tumors, human cancer cells or tumor biopsy material (patient-derived xenografts (PDXs)) are grafted to immunocompromised mice. In the past two decades, PDX models were used as a main preclinical screening tool when developing new methods to treat cancer. The models helped to identify the clinically effective chemotherapeutic agents and are still the most common in pharmaceutical industry [2], primarily because the effects of various agents on tumor cells and tissues are possible to rapidly and easily evaluate in vivo with the models [1]. Construction of such models for individual patients became an important step towards personalized therapy [3].

Mouse strains used to construct PDX models. Only immunodeficient or immunocompromised mice can be used to graft human cells and tissues. A panel of immunocompromised mouse strains was developed for the purpose. The most common strains are listed below according to the degree of immune impairment. Athymic nude mice carry a spontaneous deletion of the *Foxn1* gene. The deletion causes abnormal development or lack of the thymus, leading to suppression of the immune system and lower T-cell counts. The resulting immunodeficiency is severe, but not absolute because nude mice preserve a minor amount of T cells at the periphery and intact innate immunity with high activity of NK cells. This feature can limit the engraftment rate (percent successfully engrafted tumors) and impair the growth and metastasis of the majority of primary solid tumors and precludes the engraftment of malignant hematopoietic cells [4]. Mice with severe combined immunodeficiency (SCID) almost totally lack B- and T-cell immunity as a result of a rare genetic disorder. SCID mice show normal differentiation and function of nonlymphoid blood cells, including monocytes, granulocytes, megakaryocytes, erythrocytes, and NK cells [5]. Greiner and colleagues [6] constructed NOD/LtSz-SCID mice, which are good recipients of human cells. High engrafting rates of human cells in NOD/LtSz-SCID mice are explained by multiple immune dysfunction, which includes a decrease in macrophage function, lower complement-dependent hemolytic activity, and impaired activity of NK cells [6]. However, residual NK cell activity is preserved in these mice [7]. To overcome the problem, the mouse strains NOD/SCID/$\beta2m^{null}$ and NOD/SCID/γ_c^{null} were designed to have pronouncedly lower production of interferon γ (IFN-γ) in dendritic cells. The two strains are fully devoid of NK cell activity and consequently ensure high engraftment rates of human cells [7, 8]. The variants NOD.Cg-Prkdc^{scid}Il2rg^{tm1Sug} (NOG), NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjll} (NSG), and NOD.Cg-Rag1^{tm1Mom}Il2rg^{tm1Wjl}/SzJ (NRG) were additionally constructed as immunodeficient IL2rg^{null} mice on the basis of the NOD strain. NOG and NSG mice carry a mutation of *Prkdc*. NOG mice have a shortened cytoplasmic domain in the common γ chain of the interleukin 2 receptor (Il2rg); the γ chain is deleted in NSG mice; and NRG mice have target *Rag1* mutations that impair the function of the encoded protein [9]. Both innate and adaptive immunity is critically distorted as a result of these modifications in NOG mice. NSG mice lack T, B, and NK cells and ensure high engrafting rates upon transplantation of human hematopoietic stem cells from the umbilical cord blood [10]. More detailed discussion of the features of the models is out of the scope of this review.

PDX models of EBV infection. PDX models of virus-associated tumors were obtained for several viral infections, starting from EBV infection. In 1999, Murakami et al. [11] were the first to demonstrate that epithelial cell lines infected with EBV, which belongs to the herpesvirus family, produce tumors when grafted to SCID mice. The EBV-positive epithelial cell lines GT38 and GT39 were obtained from nonmalignant human gastric carcinoma regions and implanted subcutaneously to SCID mice in these experiments. Tumors developed in all of the mice within approximately 2 months after grafting. The tumors were identified as undifferentiated carcinoma and had detectable levels of the circular, but not linear, EBV DNA, which expressed EBV-encoded small RNA-1 [11]. Similar experiments were carried out with conditionally immortalized endothelial human cells (HuARLT) permissive to infection with Kaposi's sarcoma-associated herpesvirus (KSHV). Cells infected in vitro before grafting or in vivo after engraftment produced tumors in immunodeficient mice ($Rag2^{-/-}\gamma c^{-/-}$) [12]. More recent studies showed the development of EBV-associated lymphocytic tumors from colorectal, gastric, breast, and lung cancer samples grafted subcutaneously to NOG mice [13]. Tumors consisting of EBV^+ $CD45^+/CD20^+$ B cells grew rapidly and formed large metastatic lesions in lymph nodes, liver, lungs, and spleen of mice, while primary carcinoma grafts grew slowly without metastasis [14]. To summarize, EBV^+ lymphocytic xenografts provide a convenient model to study EBV-associated tumors.

PDX models of HPV infection and cervical cancer. A panel of cervical cancer (CC) models was constructed by xenografting patient-derived CC samples (CC-PDX). In total, 61 CC-PDXs were obtained from 98 patients with an engraftment rate of up to 75% [15]. Many characteristics of primary tumors were preserved in CC-PDXs, including genomic and histological features, sensitivity to anticancer drugs, and even morphological features (staining with hematoxylin and eosin). For example, centers of mitotically active cells, structurally similar collagen deposits, patterns of cytoplasmic immune staining for HPV, and widespread nuclear staining for p16INK4a were observed in primary biopsy samples and xenografts obtained after serial grafting [15, 16]. Higher successful implantation

rates were achieved when severely immunodeficient mice, such as SCID, NOD, or NSG mice, were used to obtain CC-PDX models [15].

PDX models of HBV and HCV infections. PDX models make it possible to study the clinical and pathological features of HBV infection. A Trimera model was the first one, in which human liver fragments were infected with HBV ex vivo and grafted to SCID mice [17]. Engraftment of the human liver fragments was assessed by hematoxylin–eosin staining and expression of the human serum albumin mRNA and was successful in 85% of the transplanted mice 1 month after grafting. HBV DNA became detectable 1 week after liver transplantation, and viremia peaked 2–3 weeks after transplantation. The HBV-Trimera model was used to evaluate the therapeutic effects of human polyclonal anti-HBs antibodies and reverse transcriptase inhibitors, which were found to decrease both the percentage of infected mice and the viral load in their sera [17]. A unique panel of hepatocellular carcinoma (HCC) samples and respective HBV-positive stromal tissues was transplanted to NOD/SCID mice in a more recent study [18]. All HCC cells were found to produce HBcAg and displayed high proliferative activity, while only benign hepatocytes tested positive for HBsAg. The HBV/HHC models in mice phenocopied the virological and cell features of patient tissues. The study showed that PDX models are suitable for studying HBV and HBV-induced carcinogenesis [18].

A PDX model of HCV-associated HCC was similarly developed in mice with severe immunodeficiency [19]. Primary tumor tissues from the liver of a patient with chronic hepatitis C were transplanted to NSG mice, and a xenograft tumor culture was established after several in vivo passages. The patient primary tumor and mouse xenografts were histologically similar. Genetic profiling confirmed the identity of HCV-HCC-PDXs and the clinical sample. Human albumin, α1 antitrypsin, glypican 3, α-SMA, and type 1A2 collagen markers were detected in original human tumor tissues and xenografted tumors. The HCV-HCC-PDX model was successfully used to test chemotherapeutic agents for efficient treatment of HCC. The model is still promising for evaluating new or accessory targeted anti-HCC agents. However, it is noteworthy that the HCV RNA became undetectable during passaging, unlike in the HBV-HCC-PDX model [19]. The HCV-HCC-PDX model is therefore unsuitable for investigating HCV, the mechanisms of HCV-associated carcinogenesis, and immunotherapy of HCV-associated HCC.

Advantages and drawbacks of PDX models. In general, PDX models are similar to primary tumors in histological features, including characteristics of the cell genome, the status of the microenvironment, and intertumor and intratumor heterogeneity [20]. Their sensitivity to chemotherapeutic agents is similar to the sensitivity observed upon clinical use of anticancer drugs. PDX-based mouse avatars are now intensely developed to optimize therapy for chronic viral and oncology diseases in humans and to allow personalized therapy [21]. The engraftment rate may be comparatively low (e.g., see [15]), but is possible to increase by using immortalized or cancer cell lines derived from patient tissues [16]. PDX models can thus been used at various steps of improving diagnosis and treatment of cancer and in concurrent preclinical/clinical trials to evaluate the treatment efficacy, to overcome drug resistance, to identify the biomarkers, and to achieve precision medicine [20].

At the same time, several drawbacks and limitations are characteristic of PDX models compared with autochthonous mouse models of cancer. First, suppression of the immune system in mice excludes the use of murine models in studies of the efficiency of vaccines and the sensitivity of immunotherapy. Second, the immunocompromised status of PDX models does not allow them to adequately reflect the process of tumor growth and, especially, metastasis because inflammation, which is determined by immune cells, plays a substantial role in metastasis [16]. A limitation specific to xenografts of immortalized or transformed human cell lines consists in the fact that a line results from expansion of a certain clonal component of a polyclonal tumor because cell or tissue explants are under selective pressure exerted by culturing-related processes [22]. Features of autochthonous tumors may be changed or completely lost in xenografts as a result of these processes. The features include surrounding normal tissues, stromal cells, vascular and lymphatic circulation, and immune cell infiltration (for a detailed review, see [1, 23]). Predictive potential of xenograft models is questionable because of these specifics and limitations and is variously estimated both as sufficiently high [24] and as critically low [25].

Several practical drawbacks are additionally characteristic of the models. For example, progression to EBV^+ and highly metastatic lymphocytic tumors was observed for a substantial portion of PDXs obtained from patients' heterogeneous tumor samples, including breast, colorectal, pancreatic, bladder, and kidney cancers. Their progression jeopardizes the experimental results [14] and warrants a systematic testing of human tumor tissues and cells prior to grafting in order to exclude contamination with extraneous viruses (EBV in this case). Experiments with HBV-HCC-PDX showed additionally that the intensity of cell proliferation in xenografts is substantially higher than in original human tissues (as assessed by ki67 staining), indicating that a cancer cell subpopulation with a higher propensity to proliferate in mice may be selected during engraftment [26], leading eventually to a difference between PDX and original tumor tissue. Another factor is the last, but not the least. Only transient viral expression in xenografts was observed in experiments with HCV-HCC-PDX, limiting the applicability of PDX models for studying viral infec-

tions and virus-associated carcinogenesis, at least as far as HCV is concerned.

Human Tissue Xenografts with Artificial Virus Infection or Production of Particular Viral Proteins

In parallel with constructing patient tumor-derived xenograft models, models were designed where immortalized or transformed human cells were artificially infected with a virus before being grafted to mice or were first grafted and then infected with a particular virus. Models of this kind were experimentally constructed for high-risk HPV-, HCV-, and HBV-associated tumors.

To model the pathologies associated with high-risk HPVs, artificial human skin was obtained with the use of primary keratinocytes producing the E7 oncoprotein of HPV 16 and grafted to nude mice. The grafts stably produced E7 for 6 months after transplantation. The transplanted mice developed lesions that histologically resembled the human anogenital lesions caused by carcinogenic HPVs. Immune detection or quantitative PCR of the HPV mRNA main biomarker microRNAs showed that the skin grafts modified with HPV 16 E7 has common molecular characteristics with pretumor and tumor lesions associated with high-risk HPV infection in humans [27]. The model provided a valuable platform for basic studies of HPV-associated carcinogenesis.

First achievements were made in the case of HCV as well. A panel of tumor xenograft models was constructed for HCV infection in SCID mice. The panel utilized the human hepatocytes that were adapted to mice and carried genomic or subgenomic HCV replicons and reporter genes for luciferase or the green fluorescent protein (GFP) [28, 29]. Cell lines implanted to SCID mice produced subcutaneous tumors and liver metastases, which replicated HCV. Tumorigenesis was similarly induced in immunodeficient mice by grafting Huh7 cells, which originate from human hepatocellular carcinoma, and the mice were then infected with HCV [30]. Each of the variants makes it possible to study HCV replication and to test antiviral drugs against HCV, but not to test vaccines and to study the immunopathogenesis of chronic HCV infection, including HCC development (because B- and T-cell immune responses are absent in SCID mice). Similar models were constructed with HBV and transgenic SCID mice expressing the urokinase-type plasminogen activator (uPA/SCID mice) (see *Transgenic Mice with Patient-Derived Xenografts* below).

Use of cell lines made it possible to overcome the above practical drawbacks of PDX models, such as contamination with heterogeneous viruses or tumors, selection of individual clones differing in properties from other cells, and spontaneous clearance of viral infection. However, the approach fails to allow a testing of vaccines and immunotherapy methods, as is the case with PDX models.

Grafting of Human Immune System Elements

Human immune cell grafting can further improve the above models and to solve the immunodeficiency problem at least in part.

Several humanized murine models were constructed using immunocompromised mice with human immune cell grafts.

The hu-PBL-SCID (human peripheral blood lymphocyte) model was obtained by grafting human mature peripheral mononuclear cells (predominantly $CD3^+$ T cells) from blood, spleen, or lymph nodes.

The hu-SRC-SCID (human SCID repopulating cell) model was obtained by grafting hematopoietic stem cells, which allowed the development of hematopoietic and naïve immune human systems.

The SCID-hu model was obtained by grafting human fetal liver and human fetal thymus. The mice develop a new hematopoietic focus and a new organoid that performs the functions of the human thymus.

The hu-Tg model express human transgenes.

Thus, new-generation hu-mouse models reproduce maturation of a broad range of human hematopoietic cells, including T cells, B cells, macrophages, and dendritic cells, which are all necessary for producing a specific immune response. Both infection and the immune response to infection are possible to reproduce with these models [31]. The models are suitable for studying tumors [2, 32, 33]; autoimmunity [34]; and human-specific virus infections, as was demonstrated with HIV-1 [35], Dengue virus, EBV, Kaposi sarcoma virus [36–38], and several other virus infections [34].

EBV infects exclusively human B cells and has a biphasic latent cycle and a lytic cycle. An adequate murine model is difficult to design because of these features and the fact that both innate and adaptive immune responses are involved in fighting infection. Humanized mice with T-cell immunodeficiency are broadly used in the case of EBV. In these models, EBV induces lethal lymphoma with a type III latent EBV gene expression pattern, which is similar to post-transplantation lymphoproliferative disorders. The models are used to test vaccines, to study adaptive cell approaches, and to develop new therapeutic strategies. The hu-PBL-SCID model was widely used in preclinical studies of anti-EBV therapies (for a review, see [39]). A new generation of humanized mice (NOG and NSG) with a reconstructed human immune system made it possible to construct unique immunocompetent models of EBV infection. The models help to study EBV-induced malignancies and means to prevent their development (for a review, see [39]).

Similar models based on hu-PBL-SCID mice were used to investigate passive immunization against HIV-

1. Repopulation with human immune cells renders immunodeficient mice susceptible to infection with HIV-1, which is a lymphotropic virus [40, 41].

Still the models fail to fully correspond to immunocompetent animals and are rather expensive, which limits their broad use.

TRANSGENIC MICE EXPRESSING HUMAN GENES

Use of transgenic humanized mice expressing human genes makes it possible to overcome the limitations due to immunodeficiency and to shed light onto the context of virus-induced carcinogenesis in vivo in the presence of a functional immune system. Mice are often used because they are genomically and physiologically similar to humans and are easy to modify genetically. In particular, transgenic mice are used to model the progression of viral infections and infection-associated disorders. Because the immune system is intact in transgenic mice, they are suitable for studying both chemotherapeutic and immunotherapeutic agents [42].

Various methods can be used to construct transgenic mice, including the Cre and Tat recombinase systems, standard and conditional knockouts, RCAS (replication-competent ALV LTR with a splicing acceptor), etc. [43].

It is of immense importance to choose a proper original strain when constructing transgenic mice because the genetic background may perform both positive and negative roles. For example, the C3H/HeN(C3H) strain has a higher frequency of spontaneously and chemically induced HCC as compared with the C57BL/6 strain, although hindering the interpretation of data on the effects of a carcinogenesis-related gene, while the C57BL/6 strain has a relatively low frequency of spontaneous tumors [44]. Hyperplastic or dysplastic lesions developed in 100% of mice in models based on the C57BL/6, BALB/c, and SSIN/SENCAR strains that expressed early HPV 16 genes under the control of the keratinocyte K14 gene promoter. However, only mice resulting from backcrosses with the FVB/n strain developed malignant squamous cell carcinomas of two grades, highly and moderately/low-differentiated, which each displayed characteristic patterns of malignant behavior [45].

Mice Transgenic for Factors That Allow Infection with Human Viruses

Viruses that are pathogenic for humans are not always capable of infecting mice. The problem is possible to solve by constructing mice that express the respective human virus receptor transgene, which confers susceptibility to viral infection on mouse cells. Both in vitro and in vivo models were obtained on this basis to study viral infections and virus-associated pathogenesis.

Transgenic mice that express the human angiotensin I-converting enzyme 2 (ACE2) receptor gene under the control of the cytokeratin 18 (K18) gene promoter (K18-hACE2) provide a model to study SARS-CoV-2 infection. Intranasal inoculation of SARS-CoV-2 to K18-hACE2 mice leads to high-level virus replication in the lungs and a spreading of infection to other organs [46]. The human poliovirus receptor (PVR) gene was used to construct transgenic mice that express PVR transcripts and poliovirus-binding sites in a broad range of tissues. Intracerebral injection of type 1 poliovirus (strain Mahoney) to PVR-transgenic mice led to viral replication in the brain and spinal cord and the development of paralytic poliomyelitis [47].

However, the presence of viral receptors on the cell surface is not the only factor that determines mouse susceptibility to human viruses. Genetic differences between mice and humans result in differences in viral replication, susceptibility to certain viruses, and pathological features of viral infections between the two species. Comparative sequencing of the human and mouse genomes revealed 300 unique genes, but only 40% of the mouse genome aligns with the human genome at the nucleotide level [48]. Replication of viruses as obligatory intracellular parasites depends on the function of host systems and host cell metabolism, which differ in many features between human and mouse. Many host cell factors involved in virus replication make this pathway extremely difficult for the majority of viruses. For example, infection of human hepatocytes with HCV depends on the surface virus entry factors CD81, occludin (OCLN), the tight junction protein claudin-1 (CLDN1), and scavenger receptor class B type 1 (SR-BI). CD81 and OCLN are absolutely essential for mouse cell infection [49]. However, infected mouse cells still fail to sustain HCV replication and chronic HCV infection because of the differences in many factors that determine viral replication in human cells. The factors include the low density lipoprotein receptor, glycosaminoglycans, ephrin receptor A2, receptor tyrosine kinases, the epidermal growth factor receptor, cadherin E, cell death-inducing DFFA-like effector B, Niemann–Pick C1-like protein 1, and transferrin receptor 1 (for a review, see [50]). Exogenous human *NTCP* expression provides another example, conferring susceptibility to hepatitis D virus (HDV) on the originally unsusceptible cell lines HepG2 (human), Huh7 (human), Hepa1–6 (mouse), and AML-12 (mouse) and primary mouse hepatocytes (PMHs). HDV utilizes the HBV envelope proteins. Human NTCP determines HBV sensitivity only in the HepG2 and Huh7 human cell lines, but not in the Hepa1–6 and AML-12 mouse cell lines and PMHs. These findings indicate that human NTCP acts as a functional receptor to mediate HBV infection of human cells, but is incapable of sustaining HBV infection in mouse hepatocytes and that other intracellular factors are required for mouse

hepatocyte infection [51]. HIV-1 replication in human cells depends on a number of factors, including cyclin T1, the splicing inhibitor p32, APOBEC (apolipoprotein B mRNA-editing enzyme, cytosine deaminase), Fut-2, TRIM5α, Lv-1, Ref-1, and cyclophilin A. Lack of these factors precludes HIV-1 replication in mouse cells [52].

Moreover, there are many differences in structure and functions of the innate immune system between human and mouse, and the differences affect the viral infection process. This primarily concerns the balance of leukocyte populations, the structure and functions of defensins, Toll-like receptors, inducible NO synthases, the cytotoxicity-inhibiting receptors Ly49 and KIR, the Fc receptor, the immunoglobulin subset, components of B-cell (BLNK, Btk, and λ5) and T-cell (ZAP70 and the common γ chain) signaling cascades, Thy-1, γδ T cells, cytokines, chemokines, cytokine and chemokine receptors, Th1/Th2 polarization, production and functions of costimulatory molecules, and antigen-presenting functions of endothelial cells [53]. The factors act together to determine the antivirus immune response and, accordingly, the possibility and specifics of virus replication in mouse cells compared with human cells. Moreover, there may be differences between different laboratory mouse strains. For example, laboratory mouse strains susceptible or resistant to influenza A virus differ in *Mx* [54]. The *Mx* gene is regulated by interferon and plays an important role in the innate immune response to influenza A virus.

Mice Transgenic for Expression of Viruses or Individual Viral Genes

If mice are impossible to infect with a human virus even with expression of a necessary receptor, it is still possible to construct transgenic mice that express particular viral genes or a total viral genome. The majority of currently known transgenic mice of the kind were obtained to study the mechanisms whereby liver pathologies are induced by hepatotropic viruses.

The full-length HBV genome or its specific subgenomic fragments were introduced in the pronuclei of a fertilized single-cell mouse embryo via microinjection. The resulting mice are incapable of eliminating the virus, but can be used to study the HBV-induced immune response and HBV-associated carcinogenesis. HCC was observed to develop in transgenic mice with stable production of HBx, full-size HBsAg, and pre-S mutants [55, 56]. Studies with these models demonstrated that viral carcinogenic proteins play an important role and act alone or in combination with human oncogenes to facilitate HCC development by inducing oxidative stress, altering the regulation of host gene expression, and activating the carcinogenic signal transmission pathways [56]. HBV-transgenic mice were additionally used to study the effects of drugs (including cytokines) designed to prevent HBV progression and to develop strategies to overcome immune tolerance to HBV [55].

To study the effects of HCV on liver pathology, steatosis, and HCC induction, transgenic mice were similarly constructed to produce the full-size HCV polyprotein or particular HCV proteins [54]. Transgenic mice that produce the nucleocapsid (core) proteins of HCV were used to analyze the mechanisms of HCV-induced carcinogenesis. Long-term production of the HCV core protein was found to trigger HCC development in transgenic mice. HCC develops possibly because the HCV nucleocapsid protein alters the oxidant–antioxidant balance in the liver without inducing inflammation [57]. However, it remains unclear whether the data obtained with the models can be used to explain the pathologies observed in humans. It should be noted that overproduction of HCV proteins is characteristic of transgenic mice and dramatically differs from low levels of HCV proteins observed in natural infection. Expression of the HCV genome in mouse cells does not result in production of virus particles or replication of the viral genome, in contrast to HBV-transgenic models. These models are therefore unsuitable for studying viral infection and modeling the total natural process that involves cell and body infection and virus spreading and is observed in human tissues [54].

Another limitation of the above HBV and HCV infection models is that the virus is expressed in all tissues of the body, while natural infection occurs predominantly (HCV) or exclusively (HBV) in the liver. Taken together, these properties render HBV- and HCV-transgenic models unsuitable for evaluating antiviral drugs and therapeutic or immunotherapeutic methods and developing methods to control the infection process, in particular, histological monitoring of viral clearance in liver tissues [58]. The limitations are possible to overcome in part by using an exogenous organ- or tissue-specific promoter to control expression of the virus (or viral genes). For example, the constitutive promoter of the albumin gene and the inducible promoter of the metallothionein gene determine transgene expression in the liver [56].

Several attempts were made to construct transgenic mice that express the full-length HIV-1 genome [52, 59, 60]. For example, transgenic mice were engineered to carry intact HIV-1 provirion copies. The mice did not display signs of infection during a 9-month observation period. However, one mouse produced offspring (F1), which developed a disease similar to the acquired immunodeficiency syndrome and died on day 25 after birth. HIV-1 undistinguishable from the parental virus was isolated from the spleen, lymph nodes, and the skin of the affected mice (five out of five) [59]. Transgenic mice that carry individual HIV-1 genes, e.g., *tat*, were also constructed [61, 62]. Like other models, mice transgenic for individual viral genes lacked both receptors necessary for viral infec-

tion and cofactors necessary for efficient viral replication and were tolerant to production of the viral proteins. Thus, none of these models was suitable for investigating HIV-1 replication and developing new strategies of antiviral therapy or new approaches to vaccination [54].

A panel of transgenic mice that produced KSHV proteins was designed to study the oncogenic properties of the proteins. The genetically modified mice each produced one or several KSHV proteins that displayed oncogenic properties in cultured cells. The mice provide new opportunities to study the KSHV pathogenesis in order to understand the mechanisms of KSHV-associated carcinogenesis and to evaluate the efficacy of treating KSHV-associated infections by directly affecting the respective viral proteins [63].

In K14E7 transgenic mice, the HPV *E7* gene is expressed in keratinocytes under the control of the K14 promoter. *E7* expression led to hyperplasia of the epithelium, immune cell infiltration, and immunomodulation in the model, the signs being similar to HPV-associated neoplasia in humans. The signature of *E7* mRNA expression in the skin was similar to the signature of human cervical intraepithelial neoplasia stage 3. Skin samples of K14E7 mice were transplanted to wild-type mice and mice that were transgenic for the T-cell receptor and had higher amounts of E7-specific cytotoxic T cells (E7TCR269). Interestingly, the skin samples from K14E7 mice were engrafted, while similar samples of ovalbumin-expressing skin were rejected. Good engraftment of K14E7 skin transplants was attributed to expression of HPV 16 E7, which causes immunosuppression and, in particular, inhibits interferon γ expression and decreases activities of NK and mast cells [64], which are involved in transplant rejection.

MHC-Humanized Mice

Classical main histocompatibility complex (MHC) class I antigens are trimeric molecules and are found on the surfaces of nucleated cells in all jawed vertebrates. MHC-I molecules are recognized by receptors of two families: clonotype T-cell receptors, which are expressed on the surfaces of $CD8^+$ cytotoxic T lymphocytes (CTLs), and monomorphic receptors, which are expressed on both NK cells and CTLs. Production of MHC-I molecules in the cell is a consecutive process and involves many proteins, including proteases, chaperones, transporters, and certain other proteins.

Although human and mouse MHC-I molecules are highly homologous in structure, organization, and functions, there are differences in the mechanisms of antigen processing and presentation in the MHC-I context between human and mouse. Transgenesis and knockout/knock-in technologies make it possible to add certain human genes or to substitute mouse genes with their human orthologs to construct immunologically humanized mice [65]. These experimental animals are of special importance as preclinical tools to identify the epitopes in peptides presented in the HLA I context in infections and cancers and to comparatively evaluate the efficacy of immunotherapy. In models that preserved intact mouse MHC-I molecules, cytolytic responses were not limited to human HLA I-restricted responses. To better utilize transgenic HLA I molecules and to generate more functional HLA-restricted responses of $CD8^+$ T cells, Pascolo et al. [66] constructed H-2 Db β2-m double knockout mice expressing a chimeric HLA-A*02:01 α3-H chain covalently linked to human β2-m through a peptide linker (HDD II mice). Cytolytic responses were mediated exclusively by the HLA-A*02:01 monochain in the mice. To further optimize the models with transgenic HLA I molecules, Boucherma et al. [67] engineered seven new transgenic mouse strains to express the HLA I monochain in the context of a H-2 Kb, Db, β2-m triple knockout (H-2 I null). In these mice, the α1α2 H-chain domains of the HLA molecules most commonly found in all human populations (HLA-A*01:03, -A*24:02, -B*08:01, -B*27:05, -B*35:01, -B*44:02, or -C*07:01) were fused with the mouse α3 domain and covalently linked with human β2 microglobulin.

Other completely MHC-humanized mice were obtained more recently to study the HLA-restricted peptide recognition without interference of epitope presentation in the context of mouse MHC molecules [68]. In the humanized A2.DR1 model, the HLA molecules most common in Caucasians (HLA-A*0201: the epitope-binding domains α1 and α2 of HLA-A*0201 with the α3 domain of $H\text{-}2D^b$, covalently bound with human β2m (HDD)) and HLA-DR1 are expressed, while all murine MHC genes are not as a result of a knockout. The model was used to test vaccines against HPV 16; HPV infection was modeled by grafting syngenic cell lines expressing the E6 and E7 proteins [68]. For example, a transgenic cell line oncogenic for HLA-A*0201 mice was obtained by transfecting heart and lung fibroblasts from HLA-A*0201-transgenic mice with the *H-Ras* V12 oncogene and the *E6* and *E7* genes of HPV 16. The HPV 16 E7 protein was preliminarily modified to remove the dominant epitope that is presented with $H\text{-}2D^b$ to the murine immune system, thus ensuring that the anticancer immune response targets exclusively the HLA-A*0201-restricted epitopes [69]. The model was used to evaluate the efficacy of two candidate vaccines against HPV 16, which are based on plasmid DNA and a recombinant Venezuelan equine encephalitis virus [69].

Transgenic Mice with Patient-Derived Xenografts

The approach was used predominantly to model infections with hepatotropic HBV and HCV (for more detail, see [70]). The most common model is based on urokinase-type plasminogen activator-transgenic

SCID mice (Alb-uPA/SCID) or uPA/recombinant activator gene-2 (RAG-2)-transgenic mice with acute or subacute liver failure in newborns. The liver is possible to restore in these mice via its gradual repopulation with human hepatocytes [71, 72]. Severe liver failure is necessary to ensure that human cells have proper space and conditions to proliferate and to achieve high-level chimerism. Human hepatocytes remain functional for at least 2 months after transplantation and after multiple cell divisions, as was demonstrated by production of human albumin. Mice with transplanted human hepatocytes were inoculated with HBV isolated from human serum samples or an in vitro culture. The inoculation led to productive infection with viremia of up to ~10^{10} copies/mL, the formation of functional covalently closed circular DNA (cccDNA), and virus spreading [72]. The viral load increased with the increasing portion of mouse liver cells replaced with human cells, and it was possible to control both the severity of liver failure and the degree of liver restoration. Viremia continued for up to 22 weeks after inoculation (until death or a scheduled end of the experiment). The presence of infectious HBV in mouse sera was demonstrated by virus passaging. Experiments showed that HBeAg is indispensable for active virus production and transmission [73]. Thus, chimeric mice can be directly infected with HBV virions, leading to the production of functional HBV cccDNA and subsequent virus spreading. In a similar experiment, human cell lines were injected in the spleen in $FAH^{-/-}RAG2^{-/-}IL2R\gamma^{-}$ (FRG) or thymidine kinase (TK)-transgenic NOG (TK-NOG) mice, which were then infected with HBV (for more detail, see [70]). HBV infection is similarly possible in a model obtained by injecting human $CD34^{+}$ hematopoietic stem cells and human hepatocytes into the liver in newborn HLA-A2-transgenic NOD/SCID/$IL2R\gamma^{-/-}$ (A2/NSG) mice [70]. Because of the immunodeficient status of recipient mice, none of the models can be used to study immunomediated inflammation, to assess the innate or adaptive antiviral immune response, or to test immunotherapies designed to treat HBV infection or HBV-associated cancer.

To overcome this limitation, dual-humanized chimeric mice were constructed as a model including transplantation of human immune cells. Human bone marrow mesenchymal stem cells (hBMSCs) were grafted to $Fah^{-/-}Rag2^{-/-}IL\text{-}2R\gamma c^{-/-}$ SCID (FRGS) mice with fulminant hepatic failure to yield a liver and immune cell dual-humanized hBMSC-FRGS model [74]. The hBMSC-FRGS mice showed intense proliferation and transdifferentiation of functional human hepatocytes and a broad range of immune cells, including B cells, T cell, NK cells, dendritic cells, and macrophages. After HBV infection, the mice developed stable viremia and specific immune and inflammatory responses. Progression to chronic hepatitis and liver cirrhosis was observed in 55% of the mice after 54 weeks [74]. In general, the hBMSC-FRGS mice were found to provide a unique system for studying the interactions of the virus with the host organism, including pathophysiological damage to the liver in chronic hepatitis with progression to cirrhosis and specific immune and inflammatory responses of the human body to HBV infection and liver damage. The model reproduced the disease-associated gene expression profile, the cell-dependent immune response, cytokine production, and biochemical and pathological alterations characteristic of hepatitis B [74]. Mouse models AFC8-hu HSC/Hep and A2/NSG-hu-HSC/Hep were constructed in a similar study by transplanting human $CD34^{+}$ hematopoietic stem cells and human hepatocyte progenitors (or fetal liver cells) to A2 transgenic mice. In each of the models, mice could be infected with HBV, which persisted for at least 4 months [70, 75] and induced liver fibrosis. The models proved very useful for studying the mechanisms of liver damage in HBV infection [75]. Trimera models and models based on uPA/SCID and $Fah^{-/-}Rag^{-/-}IL\text{-}2^{-/-}$ mice were successfully used to study HCV infection and to develop antiviral drugs effective in treating HCV infection [76–79].

Limitations of Transgenic Mouse Models

Chimeric transgenic models have several limitations, including genetic variability, lack of tools to study the immune response, a long study duration, sophisticated laboratory methods, and ethical aspects. It is also important to note that low potential to produce tumors and metastasis and a low level of mutations in tumors are characteristic of transgenic mice [80]. Moreover, constitutive synthetic promoters are most often used to control foreign gene expression in transgenic mice. Gene expression cannot vary depending on the genetic background of a model with such a promoter, leading to low cell heterogeneity and affecting the character of tumor progression and metastasis [81].

Construction of murine models to study human virus infections is developed well enough for viruses that utilize a limited set of host cell factors. However, even these viruses replicate in mouse cells to a lower level than in human cells, the difference being associated to a great extent with activation of innate immunity and production of type I interferons [82]. Molecular sensors of nucleic acids and IFN I suppress virus propagation and block the development of the signs of infection in the majority of models, thus complicating the study of virus-induced pathology.

As mentioned above, the immune system essentially differs between human and mouse. Murine models therefore fail to exactly reproduce the human immune response to viruses or individual viral antigens, and results obtained with murine models are therefore difficult to translate to humans [82]. In view

of this, models based on humanized chimeric mice are highly promising for simulating chronic infections by hepatotropic viruses, such as HBV and HCV. Yet these models, like PDX models, are unsuitable for investigating inflammation, studying the innate or adaptive antiviral immune response, and testing immunotherapies and vaccines. The intact immune system preserved in murine models is of importance not only for solving the problems of immunotherapy, but also for studying the effects of chemotherapeutic drugs because certain chemotherapeutics were found to induce the anticancer immune response [83].

Dual-humanized chimeric models seem to overcome the problem. However, innate and adaptive immune responses are limited in these models, and the character of T and B cell-mediated immune responses to viral infection differs from the human immune response, mostly because of the cell composition of the liver microenvironment [70, 75]. In addition, the liver harbors fewer hepatocytes and sustains a lower level of viral replication in the dual-humanized chimeric models as compared to the human liver. The last, but not the least factor is that the models are based on highly specialized mouse strains and are technically laborious and expensive. Their construction and maintenance are time consuming and require money, well-developed infrastructure, and highly skilled researchers, thus limiting their broad use. It is necessary to design simpler systems that are suitable for modeling delayed sequels of chronic virus infection and long-term production of viral proteins and investigating their effects on gene expression regulation, metabolism of viral protein-expressing cells and naïve surrounding cells, and the state and function of innate and adaptive immunity.

MODELING OF VIRUS-ASSOCIATED CARCINOGENESIS

Implantation of Cancer Cell Lines Carrying Whole Viral Genomes or Individual Viral Genes into Syngenic Immunocompetent Mice

The simplest models of virus-associated carcinogenesis utilize mouse cancer (tumorigenic) cells that express viral genes. Cells are implanted into syngenic mice, that is, mice that have the same genetic background as implanted cells do. Models constructed with such cells to simulate virus-associated tumors are known as the allograft, or syngenic, models. Syngenic murine models (SMMs) retain the intact mouse immune system and are therefore well suitable for developing anticancer and antiviral drugs that affect the immune system, such as checkpoint inhibitors or preventive and immunotherapeutic antiviral and anticancer vaccines. Syngenic cell lines are possible to derive from spontaneous mouse tumors or to obtain using mutagenic compounds and transposons. Their subsequent modification to express one or several viral oncogenes in combination with known cell oncogenes can be achieved via stable transfection or transduction with retroviruses or lentiviruses or by gene insertion with the help of the CRISPR–Cas system. For example, a panel of lung cancer cell lines syngenic to C57BL/6 mice was obtained [80]. The respective protocol was modified for other cells, such as bladder [84] and rectal [85] cancer cells. Another approach is based on stable transfection or transduction of cancer cells with retroviruses or lentiviruses carrying one or several protein-coding genes of a virus. Expression of viral genes increases the oncogenic potential of the available tumor cell lines in certain cases [86, 87].

Many advantages are characteristic of syngenic models. In particular, cancer cells are easy to maintain and propagate in vitro before implantation into mice. The resulting tumors are consequently reproducible in terms of size, growth rate, and animal survival rate. The main limitations of syngenic cell lines are associated with their genetic homogeneity [83] and limited number; in other words, only a scarce panel of tumor cell lines can stably produce tumors in immunocompetent mice. It is also important to note that tumor progression is a complex process and that the effects of a particular viral protein or a whole virus may differ between human and mouse. Results obtained with SMMs are therefore only partly applicable to understanding virus-associated carcinogenesis in humans.

Descriptions of syngenic models are available in the scientific literature and at web sites of their manufacturers (e.g., https://www.criver.com/products-services/discovery-services/pharmacology-studies/oncology-immuno-oncology-studies/oncology-models/syngenic-mouse-models or https://www.taconic.com/resources/syngenic-cell-line-reference-database/) and are consequently not considered here in detail.

Infections with Rodent Viruses Related to Oncogenic Human Viruses

Mouse mammary tumor virus (MMTV), which belongs to retroviruses, should be mentioned first [88]. However, MMTV is not a model of a related virus, but actually an etiological agent of mammary tumors. The conclusion stems from the data accumulated to date, including the identification of the provirus, LTRs, the Gag and nucleocapsid proteins, reverse transcriptase, and the envelope protein in 13% of human mammary tumor samples [88].

Mouse papillomavirus type 1 (MmuPV1) is the best understood among viruses used to model virus-associated carcinogenesis. MmuPV1 was used to model HPV-associated squamous cell head-and-neck cancer in mice. Infection of the tongue epithelium with MmuPV1 in immunodeficient mice led to the development of high-grade squamous cell dysplasia with early signs of invasive carcinoma within 4 months [89]. In combination with the oral carcinogen 4-nitro-

quinoline 1-oxide (4NQO), MmuPV1 caused invasive squamous cell cancer of the tongue in both immunodeficient and immunocompetent mice. The resulting tumors expressed markers of papillomavirus infection and HPV-associated carcinogenesis [89]. Like HPV, MmuPV1 is sexually transmitted in immunocompetent mice [90], providing an opportunity to study the mechanism of infection and a model to evaluate the efficacy of preventive medicines. The mechanism of MmuPV1-associated carcinogenesis was found to be associated, at least partly, with MmuPV1 integration into the infected cell genome [91].

Mouse γ-herpesvirus 68 (MHV-68) is the most similar to EBV. Unfortunately, MHV-68 substantially differs from EBV, and there are differences in host immune response between the two viruses [39]. MHV-68 can immortalize mouse fetal liver cells in vitro, leading to their differentiation into plasmablasts, which, in turn, can produce tumors in nude and Rag2$^{-/-}$ mice, but not in immunocompetent C57Bl/6 mice or mice with B-cell deficiency [92].

Chimeric HIV-1/murine leukemia virus (MuLV) pseudovirions were designed to model HIV-1 infection. The chimeric virions are possible to obtain by infecting a MuLV-carrying T-cell line with HIV-1 or replacing the HIV-1 *gp120* coding region with MuLV *gp80* [52]. To evaluate the protective properties of candidate vaccines against HIV-1, mice were immunized with the vaccines and then infected with the chimeric pseudovirus [52].

In general, the approach is used relatively rarely because only few rodent viruses are similar to oncogenic human viruses.

In Vitro and In Vivo Modeling of the Molecular Background That Facilitates Cell Malignant Transformation

A more difficult task is to model the interaction between viral and human oncogenes/oncoproteins as part of virus-associated carcinogenesis. Cell malignant transformation and tumorigenesis are known to depend on the intricate interactions between viral oncogenes and host oncogenes and oncoproteins. Early evidence for their cooperation was obtained using polyomavirus oncoproteins for in vitro cell transformation. Expression of the large and middle T-antigens of the polyomavirus proved necessary for transformation of primary rat embryonic fibroblasts [93]. Similar gene cooperation is broadly used to model viral infection-associated tumors.

Viruses have to suppress or arrest apoptosis for a sufficiently long period of time to ensure maturation and release of their progenies. Oncogenic viruses are especially efficient in modulating both intrinsic and extrinsic apoptosis pathways and inhibit proapoptotic proteins and signaling cascades, thus facilitating carcinogenesis [94]. Viruses most often target p53-induced apoptosis, which is mediated by a linear cascade that involves *bax* transactivation; Bax translocation from the cytosol into the membrane compartment; cytochrome *c* release from mitochondria; and consecutive activation of caspases 9, 3, and 7 [95]. Mice with null mutations of the p53 gene and p53 deficiency showed accelerated tumorigenesis [96], and a broad panel of tumor models was accordingly constructed on the basis of p53 mutants [97]. Viruses functionally inactivate p53 to exert the same effect. Adenovirus and polyomavirus T-antigens bind and inactivate p53, thus allowing the cell to avoid a cell cycle arrest and to enter the S phase. In addition, viral T-antigens mimic the DNA structure and compete with p53 for DNA binding, changing the charge and configuration of the DNA duplex [98]. This property helps T-antigens to alter the transcriptional regulation of p53 target genes and to cause malignant transformation of various cells, including cells nonpermissive for productive virus infection [99]. Based on this mechanism, expression of the SV40 T-antigen is used as a reliable tool to construct transgenic murine models of tumors [100].

Another example is provided by high-risk HPVs, which utilize specific activities of their E6 and E7 oncoproteins to inhibit p53. E6 recruits E6AP and promotes ubiquitin ligase activity of E6AP E3, thus causing p53 degradation and decreasing the p53 level in infected cells. The E7 oncoprotein binds to the DREAM transcriptional repressor complex, thus upregulating the majority of cell cycle genes and inhibiting p53 [101]. The retinoblastoma protein (Rb), which is another tumor suppressor, also binds with high-risk HPV E7. Rb binds with transcription factors of the E2F family and inhibits expression of replication enzyme-coding genes [102]. E7 distorts the Rb–E2F interaction, and the E2F factors are consequently released in their transcriptionally active forms to stimulate replication and cell division [103]. However, these events are insufficient for triggering cell transformation. To cause transformation of human primary cells, high-risk HPVs have to cooperate with activated human oncogenes, such as RAS, a central mediator of growth factor-induced cell proliferation and differentiation [104]. RAS acts through RAF and ERK kinases of the MAP kinase signaling pathway. RAS possesses mitogenic activity, plays an essential role during the G1 phase, and is necessary for the cell to pass through the S phase [105]. Production of activated RAS leads to cell transformation in the absence of negative regulators, such as p53 and Rb [106]. This property allowed high-risk HPV E6/E7 coexpression in combination with activated RAS to become another tool for cell malignant transformation, leading to construction of many murine models of human tumors producing the E6 and E7 proteins of high-risk HPVs [107–109]. A similar transformation scenario is possible to perform with other cooperative oncoproteins, such as MYC [110].

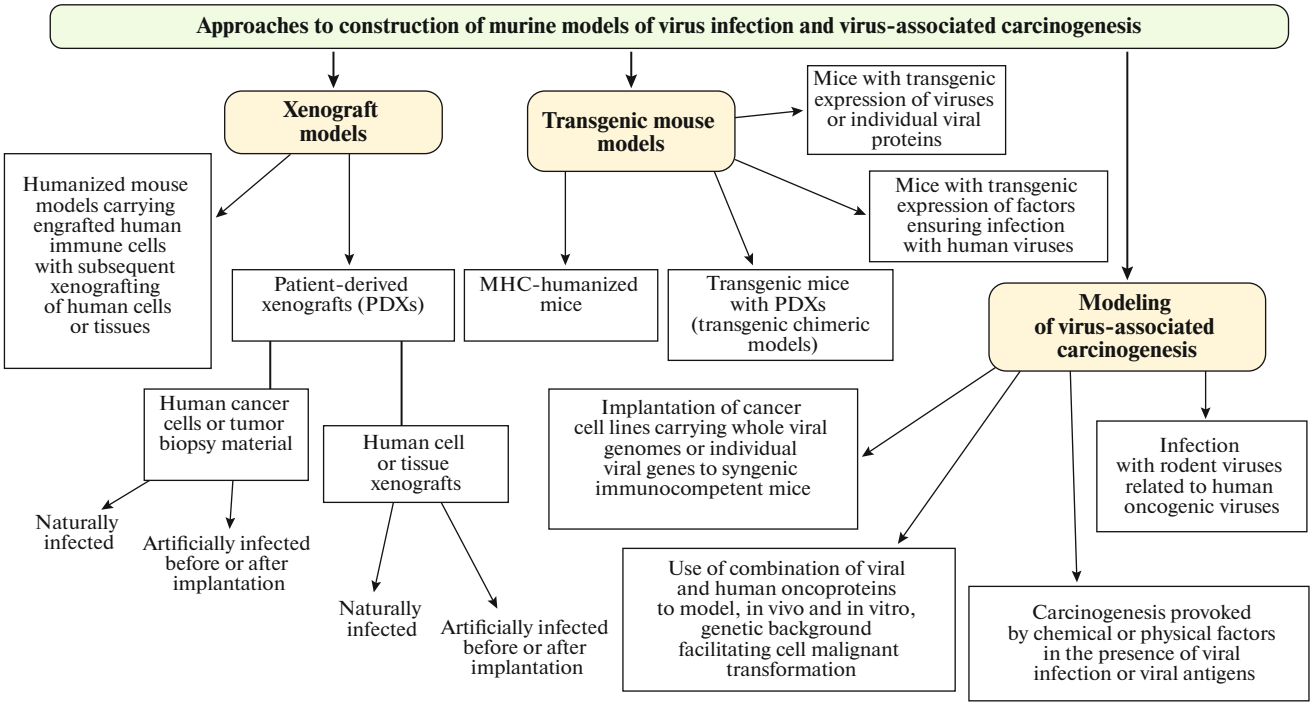

Fig. 1. Main approaches to modeling virus infections and virus-associated carcinogenesis.

Cells (cell lines) transformed under combined influence of viral and human oncogenes are implanted into syngenic immunocompetent mice. Similar syngenic murine models have been described above (see *Implantation of Cancer Cell Lines Carrying Whole Viral Genomes or Individual Viral Genes into Syngenic Immunocompetent Mice*). In vivo induction of combined carcinogenesis came to be described in the past years. A brilliant example is provided by intravaginal electroporation of mice with plasmids coding for HPV-16 E6/E7, c-MYC, AKT, and Sleeping Beauty transposase. A cooperative effect of viral and human oncogenes (oncoproteins) led to the formation of tumors, which progressed spontaneously from high-grade squamous cell intraepithelial lesions to cancer and produced HPV E6 and E7. Cell lines derived from the tumors are capable of producing carcinomas in immunocompetent mice [110]. The regulation of cyclin kinase activity was found to mediate the joint effect of the MYC and RAS cell factors and viral oncoproteins on cell transformation [106].

Models of Provoked Carcinogenesis

In this approach, various carcinogens are used to induce tumors in the presence of virus infection or expression of viral antigens; i.e., a combined effect of viral antigens and chemical or physical factors facilitates tumorigenesis. Haverkos [111] comprehensively reviewed the interactions of viruses with human carcinogens. Conclusions made in his review are applicable to murine models as well. Here we focus on the most important carcinogenesis-related factors, such as alcohol abuse, diet, and exposure to carcinogens.

Synergistic action of HBV or HCV and alcohol abuse in triggering hepatic carcinogenesis was described long ago [57]. The mechanism of HCV-associated carcinogenesis consists in the induction of oxidative stress [112]. As expected, alcohol drinking considerably increased the phosphatidylcholine hydroperoxide levels in mice transgenic for the HCV nucleocapsid protein and long-term exposure to alcohol (alcohol diet) induced liver fibrosis. The findings confirmed the synergistic effect of virus infection and alcohol abuse in the induction of liver disease [113] and demonstrated the possibility to model the process by using a combination of viral antigen expression with long-term exposure to ethanol.

Certain diets stimulate virus-associated carcinogenesis, as was demonstrated with the example of mice expressing the SV40 T-antigen under the control of the elastase 1 gene promoter. Low-fiber foods were found to increase the frequency of tumor formation [114]. It is of interest to note that a low fiber content in diet changes the composition of the intestinal microbiome; the changes lead to propagation of intestinal pathobionts, such as adherent invasive *Escherichia coli* (AIEC) associated with Crohn's disease, and subsequent changes in microbial metabolome and an increase in intestinal inflammation [115]. Systemic metabolic and immune deregulation is another consequence [116].

Table 1. Main advantages, drawbacks, and applications of various approaches to constructing models of virus infections and virus-associated carcinogenesis

Approach	Advantages	Drawbacks	Applications
Humanized mice carrying human immune cells with subsequent xenotransplantation of human cells or tissues	– Reproduce both infection and the immune response to infection	– Models do not completely correspond to immunocompetent animals. – Highly expensive	– Studying the development of malignant neoplasms. – Studying autoimmunity. – Studying human-specific viral infections. – Testing vaccines
Human cancer cells or tumor biopsy material	– Suitable for directly studying human pathogens and mechanisms of infection. – Allow rapid and simple evaluation of effects of various agents on tumor cells and tissues in vivo. – Are similar in histopathological features to primary tumors. – Are similar in chemotherapeutic agent sensitivity to human cells. – Controllable engraftment	– Unsuitable for vaccine testing. – Inadequate reproduction of tumor growth process and especially metastasis is possible because of immunocompromised status of host. – Features of autochthonous tumors may be changed or completely lost. – Contamination with irrelevant viruses is possible. – Viral expression in xenografts is transient. – Cells tend to intensely proliferate in xenografts	– Preclinical screening of new therapeutic methods (including personalized ones) to treat cancer. – Modeling the development of virus-associated cancer and metastasis
Xenografts of human cells or tissues			– Direct studies of human pathogens and mechanisms of infection. – Modeling virus-associated pathologies
MHC-humanized mice	– HLA-restricted peptide recognition is possible to study without interference of epitope presentation in the context of mouse MHC molecules	– Highly expensive. – Possibility to use HLA type 1 in every mouse strain	– Modeling immune response to viral. infection, vaccination, or immunotherapy – Studying oncology and autoimmune disorders
Transgenic mice with implanted PDXs (transgenic chimeric mice)	– Virus infection process is possible to model. – Dual-humanized chimeric mice can be used to study virus infection in the context of humanized immune system	– Genetic variability. – Lack of tools to study immune response. – Prolonged study periods. – Sophisticated laboratory methods and ethical aspects. – Low potential to produce tumors and metastasis and low level of mutations in tumors. – Models are based on highly specialized mouse strains and are highly laborious and expensive.	– Modeling chronic liver diseases, studying hepatotropic viruses and antivirus drugs

Table 1. (Contd.)

Approach	Advantages	Drawbacks	Applications
Mice with transgenic expression of viruses or individual viral proteins	– Pathogenesis of chronic viral infection can be studied in the presence of competent immune system	– Viral replication, antiviral strategies, and approaches to vaccination are impossible to study	– Studying mechanisms of virus-induced pathology. – Studying oncogenic properties of proteins in vivo
Mice with transgenic expression of factors determining infection with human viruses	– Viral infection can be studied in the presence of competent immune system	– Limited panel of host cell factors. – Fail to exactly reproduce human immune response to viruses or individual viral antigens	– Modeling all steps of infection with human viruses
Implantation of cancer cell lines carrying whole viral genomes or individual viral genes into syngenic immunocompetent mice	– Mouse immune system is intact. – Cancer cells are easy to maintain and propagate in vitro before implantation into mice – Tumors with similar growth rates and mouse survival are well reproducible	– Genetic homogeneity. – Limited panel of cancer cell lines stably forms tumors in immunocompetent mice	– Modeling tumor growth. – Preclinical screening of immune system-affecting treatments, including inhibitors of immunity checkpoints
Combination of viral and human oncoproteins used to model, in vivo and in vitro, molecular background that facilitates cell malignant transformation			– Modeling and studying virus-associated cancers
Carcinogenesis provoked by chemical or physical factors in the presence of viral infection or viral antigens	– Virus-associated carcinogenesis can be studied in the presence of competent immune system	– Poor reproducibility of effects. – Heterogeneity of effects. – Effect is impossible to restrict to single organ or tissue. – Prolonged experiments	– Studying carcinogenesis that chemical or physical factors induce in the presence of viral infection or in cooperation with viral antigens. – Studying effects that diets and inflammation exert on tumor development
Infection with rodent viruses related to human oncogenic viruses	– Viral infection can be studied in the presence of competent immune system	– Few rodent viruses are analogous to human oncogenic viruses	– Modeling human virus-induced pathologies, including tumors, in laboratory mice. – Studying mechanisms of infection. – Evaluating efficacy of preventive medicines

The same mechanisms mediates the increase induced in tumor growth by the above exposures and consists in the induction and maintenance of inflammation. The induction of inflammation underlies the well-established models of skin carcinogenesis induced with chemical carcinogens. Preexisting inflammation was shown to increase the susceptibility to tumor growth. The phenomenon is associated with an increase in the population of tumor-specific T cells producing interleukin 17 (IL-17) [117]. At the same time, IL-17 receptor (IL-17R) deficiency was observed to increase $CD8^+$ T-cell infiltration and to inhibit $CD11b^+$ myeloid cell infiltration and the development of suppressor cells of a myeloid origin [118], thus preventing tumor growth.

Tissue inflammation is possible to achieve mechanically. Isaguliants et al. [119] reported that electroporation-induced inflammation leads to a higher tumor growth in regions proximal to the electroporation site.

The above methods to model carcinogenesis associated with chronic virus infections are used to better understand the mechanisms of tumor induction and development, but not to create the respective models. This is explained primarily by the facts that the effects are poorly reproducible, heterogeneous, and are impossible to restrict to a particular organ or tissue and that experiments are rather time consuming.

CONCLUSIONS

Several standard approaches have been developed to date to create models of viral infection and virus-associated carcinogenesis and found broad application in research (Fig. 1). Each of the approaches has its specifics, advantages, drawbacks, and limitations (Table 1). These characteristics are important to consider when choosing the model. The most common approach is based on xenografts of human cells and tissues (including tumor, infected, and normal ones) with subsequent infection and transformation. The method makes it possible to rapidly obtain a panel of tools to estimate the efficacy of anticancer drugs, but suppression of the mouse immune response greatly limits its application.

Immunodeficient mice with transplanted elements of the human immune system are thought to provide the best models in the case of lymphotropic viruses, such as HIV-1 and EBV, because these models are suitable for studying the natural mechanisms of reinfection and pathogenesis for viruses of the group. However, the models are of limited utility for evaluating the efficacy of vaccines because the immune system of the model mice fails to fully reproduce the immune system of immunocompetent animals.

Transgenic animals carrying a total viral genome or individual viral genes are used to study the pathogenesis of hepatotropic viruses. These models help to study the mechanisms of virus pathogenesis, but are unsuitable for testing antiviral drugs and vaccines because virus elimination is impossible in the models.

Transgenic mice that express foreign factors essential for viral infection (receptors or intracellular cofactors) are of limited utility because a total set of cell factors is necessary to introduce in mice to allow their infection with human viruses (as is the case with HIV-1) and many factors are still poorly understood (as is the case of HCV). Modeling of virus-associated carcinogenesis with rodent viruses related to human oncogenic viruses is rarely used, primarily because the range of the rodent viruses is narrow.

Thus, although diverse murine models of viral infection and virus-associated carcinogenesis are available, there is no ideal model that completely reproduces the human processes of infection, pathogenesis, and the immune response. The model most adequate to the particular problem of interest is always necessary to choose in research.

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The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human subjects performed by any of the authors.

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