A new human retrovirus associated with prostate cancer

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Viruses and cancer have had a long history, beginning from the first discoveries of cancer viruses in animals in the late 19th century. Animal cancer viruses have provided great insights into fundamental processes of cancer, such as the discovery of cellular protooncogenes and tumor suppressor proteins (1). More recently, the involvement of viruses in human cancer has been established (2). It is now estimated that 20–25% of human cancers worldwide have a known viral etiology (2) (Table 1), although, given the multifactorial nature of carcinogenesis, other factors also contribute. In some cases, the viruses directly promote tumor development through expression of viral protein(s) that alter the growth properties of the tumor cell. For instance, the E6 and E7 proteins of oncogenic human papillomaviruses (HPVs) bind and inactivate cellular tumor suppressor proteins (3). In other cases viruses may indirectly cause cancer; the tumor cells themselves may not express the virus. Hepatitis B and C viruses appear to cause liver cancer by inducing chronic destruction and compensatory replacement of hepatocytes; the continual hepatocyte division may establish a cellular environment where other changes lead to cancer. In addition, human immunodeficiency virus (HIV) that causes AIDS indirectly causes cancer by crippling immune defenses against cancers induced by Kaposi’s sarcoma herpes virus/HHV-8, Epstein–Barr virus, and HPV 6. Viral involvements in human cancer have practical implications. First, prevention of infection may reduce the risk of developing cancer; vaccines for oncogenic HPV have recently been approved for prevention of cervical cancer. Second, mechanistic studies may identify viral or cellular targets for cancer treatment or prevention. In this issue of PNAS, Dong et al. (4) provide evidence for a new human retrovirus associated with familial prostate cancer (PC).

Studies of rare families with predispositions to cancer have led to identification of tumor suppressor genes, genes that negatively regulate cell replication or growth and whose functions are lost in the tumor cells. For instance, the Rb tumor suppressor gene was identified by studies of individuals with familial retinoblastoma (5). For many tumors, loss of the same tumor suppressor gene also occurs in similar tumors (“sporadic”) that do not have a familial association. Tumor suppressor genes have been identified by searching the genomes of high cancer families for inherited defective chromosomal regions and identifying the relevant defective gene(s). In PC, one familial susceptibility locus Hpc1 has been linked to mutations in the structural gene for RNase L (6). RNase L is an effector in the interferon induced innate antiviral response (7). dsRNA (a frequent feature of viral infections) activates 2′–5′ oligo(A) synthetase, leading to production of short oligonucleotides of 2′–5′ oligo(A). The 2′–5′ oligo(A) is a signal for the activation of RNase L, which degrades viral (and cellular) mRNAs and also induces cellular apoptosis. In Hpc1 families, a single amino acid substitution, R462Q, in RNase L leads to reduced enzyme activity. Individuals with the QQ genotype have been reported to have a 2-fold elevated risk for development of PC (7), although other studies have not confirmed this (8). The R462Q polymorphism is common (gene frequency 35%), and individuals with the QQ genotype make up ~11% of the population. The finding that Hpc1 maps to the RNase L gene suggested that inherited defects in RNase L might allow for infection with an oncogenic virus, leading to PC.

Defects in RNase L might allow for infection with an oncogenic virus, leading to prostate cancer.

In recent years, studies of the genomes of high cancer families for genes have been identified by searching for inherited defective chromosomal regions (1). It is now estimated that 20–25% of human cancers worldwide have a known viral etiology (2) (Table 1), although, given the multifactorial nature of carcinogenesis, other factors also contribute. In some cases, the viruses directly promote tumor development through expression of viral protein(s) that alter the growth properties of the tumor cell. For instance, the E6 and E7 proteins of oncogenic human papillomaviruses (HPVs) bind and inactivate cellular tumor suppressor proteins (3). In other cases viruses may indirectly cause cancer; the tumor cells themselves may not express the virus. Hepatitis B and C viruses appear to cause liver cancer by inducing chronic destruction and compensatory replacement of hepatocytes; the continual hepatocyte division may establish a cellular environment where other changes lead to cancer. In addition, human immunodeficiency virus (HIV) that causes AIDS indirectly causes cancer by crippling immune defenses against cancers induced by Kaposi’s sarcoma herpes virus/HHV-8, Epstein–Barr virus, and HPV 6. Viral involvements in human cancer have practical implications. First, prevention of infection may reduce the risk of developing cancer; vaccines for oncogenic HPV have recently been approved for prevention of cervical cancer. Second, mechanistic studies may identify viral or cellular targets for cancer treatment or prevention. In this issue of PNAS, Dong et al. (4) provide evidence for a new human retrovirus associated with familial prostate cancer (PC).

Studies of rare families with predispositions to cancer have led to identification of tumor suppressor genes, genes that negatively regulate cell replication or growth and whose functions are lost in the tumor cells. For instance, the Rb tumor suppressor gene was identified by studies of individuals with familial retinoblastoma (5). For many tumors, loss of the same tumor suppressor gene also occurs in similar tumors (“sporadic”) that do not have a familial association. Tumor suppressor genes have been identified by searching the genomes of high cancer families for inherited defective chromosomal regions and identifying the relevant defective gene(s). In PC, one familial susceptibility locus Hpc1 has been linked to mutations in the structural gene for RNase L (6). RNase L is an effector in the interferon induced innate antiviral response (7). dsRNA (a frequent feature of viral infections) activates 2′–5′ oligo(A) synthetase, leading to production of short oligonucleotides of 2′–5′ oligo(A). The 2′–5′ oligo(A) in turn activates preexisting RNase L, which degrades viral (and cellular) mRNAs and also induces cellular apoptosis. In Hpc1 families, a single amino acid substitution, R462Q, in RNase L leads to reduced enzyme activity. Individuals with the QQ genotype have been reported to have a 2-fold elevated risk for development of PC (7), although other studies have not confirmed this (8). The R462Q polymorphism is common (gene frequency 35%), and individuals with the QQ genotype make up ~11% of the population.

The finding that Hpc1 maps to the RNase L gene suggested that inherited defects in RNase L might allow for infection with an oncogenic virus, leading to PC. In a recent publication, research groups at the Cleveland Clinic and University of California at San Francisco collaborated to test this hypothesis (9). cDNAs were PCR-amplified from PCs from familial RNase L QQ patients or matched controls. They were then hybridized to the ViroChip (10) that contains conserved sequences from a wide array of eukaryotic viruses. It was striking that 40% of the PCs from QQ patients hybridized with sequences on the ViroChip corresponding to xenotropic endogenous murine leukemia virus retroviruses. In contrast, <2% of the sporadic PCs showed evidence of this virus. Using PCR-cloned cDNAs from the tumors, Ursin et al. (9) deduced the sequence for the virus [named XMRV for xenotropic murine leukemia virus (MLV)-related virus], which included a full-length potentially replication-competent retrovirus closely related to xenotropic MLVs. Viral protein was detected in the tumors, although it was in few cells, and the positive cells were stromal and hematopoietic rather than the cancer cells. Although tantalizing, these results also raised questions. In particular, the detection of XMRV was PCR-based, which raised the inevitable question of laboratory contamination. Also, because virus infection was not in the tumor cells, it was unclear whether XMRV could be causal to PC.

Retroviruses have long been associated with cancer, primarily in animals. These RNA-containing viruses replicate by way of reverse transcriptase and a DNA intermediate that integrates into the host chromosome (the provirus). Rapidly oncogenic retroviruses carry an oncogene, resulting from viral capture of a normal cell protooncogene (11). Retroviruses such as MLVs that lack oncogenes also induce tumors (more slowly) by integrating in the vicinities of cellular protooncogenes and activating them transcriptionally (11). If retroviruses infect germ cells, their proviral DNAs can be passed to progeny as stably inherited elements, endogenous retroviruses (ERVs) (12). ERVs make up substantial amounts of the genomes of many species; it is estimated that human ERVs (HERVs) and related genetic elements make up ~8% of the human genome. The mouse genome contains several families of endogenous retro-viral proviruses. Of these, endogenous MLV-related proviruses can be divided into three classes according to the abilities of their envelope proteins to mediate infection of mouse cells only (ectropic), non-mouse cells only (xenotropic), and both mouse and non-mouse cells (polytropic).

The search for human retroviruses involved in cancer has received substantial attention over the years. One human ret...
The HERV-K Rec accessory protein can be PCR-amplified from various human tumors, most notably seminomas. Moreover, HERV-K is expressed in a variety of human tumors and could be implicated in the etiology of some malignancies.

In other studies, retroviral sequences (initially designated human retrovirus-5) do not support MMTV infection. In contrast, an XMRV virus would be expected to infect human cells, given the species specificity of its envelope protein. In other studies, retroviral sequences (initially designated human retrovirus-5) could be PCR-amplified from various human tissues, but they were ultimately found to represent an ERV of rabbits (15). The possibility that expression of HERVs may contribute to tumors has also been considered. HERV-K sequences are expressed in a variety of human tumors, most notably seminomas. Moreover, the HERV-K Rec accessory protein can induce tumorigenicity in murine fibroblasts (16), and another HERV-K accessory protein (Np9) can also transform cells.

Dong et al. (4) provide key results that confirm infection by XMRV in familial human PCs. First, they assembled a complete XMRV genome from two cDNA clones and obtained infectious virus by transfecting human PC cell lines. The virus also replicates in nonprostate human cell lines. Second, they found that XMRV is sensitive to IFN, which would be consistent with the fact that it is found in PCs from patients with the RNase L QQ but not the RR genotype. Third, they showed that XMRV is using the cellular receptor for xenotropic and polytropic MLVs (XPR1) because introduction of human XPR1 into an unsusceptible hamster cell line renders it susceptible to XMRV infection. Finally, they were able to clone three junctions between XMRV DNA and human cellular DNA from two primary human PC tumors. These junction fragments confirm XMRV infection into human cells and rule out the possibility that initial identification of XMRV was a PCR artifact. Viral integration sites were localized to the genes for two transcription factors (CREB and NFAT) and a suppressor of androgen receptor transactivation (APPPB2/PAT1/ARA67); the potential involvement of these genes in PC is at least plausible. The fact that XMRV is IFN-sensitive and detected at high frequency in PCs from Hpc1 QQ families suggests that infection is quite common, at least within that patient population. (i) What is the origin of XMRV? Its homology to xenotropic MLVs suggests that it might be a zoonotic infection, i.e., infection by a virus from mice. (ii) How broadly distributed is XMRV infection in humans? The fact that 40% of QQ PCs showed infection (9) suggests that infection is quite common, at least within that patient population. (ii) Is XMRV associated with any other human cancers? MLVs in mice induce leukemias, although leukemogenicity depends on high levels of viral infection. The answers to these questions are eagerly anticipated.

Finally, the discovery of XMRV as a new retrovirus of humans reinforces the idea that other human diseases may involve infections by viruses (or other infectious agents) either known or previously unknown.

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**Table 1. Human viruses associated with cancer**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Cancer</th>
<th>Causal role established</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human T cell leukemia virus type I</td>
<td>Retrovirus</td>
<td>Adult T cell leukemia</td>
<td>Direct/indirect</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Hepadnavirus</td>
<td>Hepatocellular carcinoma</td>
<td>Direct</td>
<td></td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Flavivirus</td>
<td>Hepatocellular carcinoma</td>
<td>Indirect</td>
<td></td>
</tr>
<tr>
<td>HERK</td>
<td>Retrovirus</td>
<td>Testicular cancer, other cancers</td>
<td>No [1]</td>
<td>Direct?</td>
</tr>
<tr>
<td>MMTV</td>
<td>Retrovirus</td>
<td>Breast cancer</td>
<td>No</td>
<td>?</td>
</tr>
<tr>
<td>XMRV</td>
<td>Retrovirus</td>
<td>Prostate cancer</td>
<td>No</td>
<td>Indirect?</td>
</tr>
</tbody>
</table>

However, another possibility is that XMRV is not causal to PC but reflective of the reduced antiviral status of RNase L QQ individuals; another novel virus whose sequences were not detected by the VirusChip might be the relevant agent.

The discovery of a new human retrovirus in familial PC patients is exciting and raises several questions for future studies: (i) Is XMRV infection causal to PC in Hpc1 families, and is it involved in any nonfamilial cases? (ii) What is the mechanism of tumorigenesis? An indirect mechanism is suggested by viral expression in stromal but not tumor cells. Indeed, the role of stromal changes in PC has been described (17). (iii) What is the origin of XMRV? Its homology to xenotropic MLVs suggests that it might be a zoonotic infection, i.e., infection by a virus from mice. (iv) How broadly distributed is XMRV infection in humans? The fact that 40% of QQ PCs showed infection (9) suggests that infection is quite common, at least within that patient population. (v) Is XMRV associated with any other human cancers? MLVs in mice induce leukemias, although leukemogenicity depends on high levels of viral infection. The answers to these questions are eagerly anticipated.

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