**CANDIDATE TUMOR VIRUSES**

**Is a Retrovirus Implicated in Familial Prostate Cancer?**

By Vicki Brower

Three years ago, researchers discovered a retrovirus that appeared to be more common in the tumors of men with familial prostate cancer than in those with sporadic prostate cancer. Since then, the virus, XMRV, has become the focus of a growing number of studies in the U.S. and elsewhere. Not everyone agrees that XMRV is involved in familial prostate cancer, but there is little doubt that the discovery has attracted the attention of viral researchers.

That’s partly because XMRV is one of a very few authentic, known human retroviruses, said Stephen Goff, Ph.D., who heads a laboratory studying retrovirus replication at Columbia University in New York. Although retroviruses have long been implicated in animal cancers, only three—human T-lymphotropic viruses 1 and 2 and human immunodeficiency virus—have been linked to cancer in humans.

“Retroviruses have had a horrible, checked history, with lots of ‘rumor viruses’—mouse-contaminated cells mistaken for mouse viruses infecting humans,” Goff said. “The question is, does XMRV cause or contribute to prostate cancer, or is it merely a passenger virus?”

XMRV was first discovered by researchers at Ohio’s Cleveland Clinic and the University of California in San Francisco. “No one was looking for this type of virus in prostate cancer,” said Robert Silverman, Ph.D., professor of cancer biology at Cleveland Clinic and leader of the team that discovered the virus. Interested in innate antiviral immunity, Silverman had earlier discovered a gene, RNaseL, that protects against viruses. And he knew that men with RNaseL mutations have both reduced immunity to viruses and a higher risk of prostate cancer, suggesting that a virus could be associated with the disease. In search of such a virus, he teamed up with Joseph DeRisi, Ph.D., of the University of California in San Francisco, who had used his invention, the ViroChip—a microarray chip loaded with every known virus—to identify the SARS (severe acute respiratory syndrome) virus a few years earlier.

With the ViroChip, Silverman and DeRisi looked for a virus in prostate tumors from 86 men with prostate cancer. Of the 20 men with two copies of the RNaseL mutation, eight, or 40%, had a virus similar to murine leukemia virus. In contrast, only one of the 66 men with one or two normal copies of the gene had the virus. “We related the presence of the virus to the presence of the genetic mutation, cloned the complete genome from the virus, and produced infectious virus,” said Silverman. Because it was a xenotropic virus—one that can grow only in foreign cells other than mouse cells—they named it xenotropic murine leukemia virus–related virus, or XMRV.

The discovery excited researchers because it suggested that a small percentage of prostate cancers could have an infectious origin. “Viral involvements in human cancer have practical implications,” said Hung Fan, Ph.D., professor of molecular biology and virology at the University of California at Irvine. “First, prevention of infection may reduce the risk of developing cancer. Second, mechanistic studies may identify viral or cellular targets for cancer treatment or prevention.”

**The Questions**

The discovery opened up a new area of research with many unanswered questions. “Is XMRV found in men with familial prostate cancer because they cannot clear the virus, and is the virus a factor in their development of cancer?” asked David Griffiths, Ph.D., of the UK’s Moredun Research Institute. Why is it found in prostate cells? Is it present in other cell types, such as B and T cells, which are usually infected by retroviruses, and in other immunocompromised individuals? Is it a factor in human leukemia as well?

Scientists generally agree that molecular data suggest that the virus exists in prostate cancer tissue and is not a lab contaminant or an endogenous retrovirus, as some assert about the mouse mammary tumor virus (MMTV). (J. Natl. Cancer Inst. 2009;101:293–5). Unlike MMTV, one can distinguish XMRV on the basis of its DNA sequence, said Fan. Also, its complete genome has been assembled and an infectious, replicating virus has been produced. An assay for the virus has been developed with Abbot’s diagnostics organization, from which Silverman and co-discoverers could receive royalties.

After that initial discovery, the next step toward proving causation was to find integration sites for the virus—locations where it enters human cells. Silverman and Samson Chow, M.D., Ph.D., associate professor of pathology at the University of California in Los Angeles, discovered 14 integration sites, and they cloned the junctions between the viral and human DNA, indicating that the virus actually infects human cells. Chow is currently trying to map more sites to determine exactly how the virus might contribute to carcinogenesis.
Although it is not known how XMRV is transmitted between humans, Silverman suspects sexual transmission might be one way. His lab, with collaborators at Germany’s University of Ulm, recently showed that factors found in human semen enhance XMRV infection of cells in the lab. The German group previously showed that the same factors enhanced human immunodeficiency virus infectivity by 100,000-fold.

“If XMRV is passed from human to human, which we believe it is, the virus may infect and persist predominantly in those with defective innate immunity, and adaptive immunity may result in the presence of antiretrovirus antibodies in some patients,” Silverman said.

However, a group led by Ila Singh, M.D., Ph.D., at the University of Utah in Salt Lake City found the prevalence of different RNASel genotypes to be the same in patients with prostate cancer that test positive for XMRV as in those who test negative for the virus.

Singh’s group has confirmed the presence of XMRV in prostate cancer samples from the Cleveland Clinic. They have also found the virus in about 6% of about 250 prostate cancer samples from the Columbia University tumor bank, using a quantitative PCR-based test. Singh said her university may apply for a patent for this assay, which measures XMRV DNA, and for an immunohistochemistry method using an XMRV-specific antibody to analyze virally infected tissues.

An important difference between Silverman’s and Singh’s findings is that Singh found viral proteins expressed primarily in malignant epithelial cells, and in rare cases, in a few stromal cells adjacent to the malignant cells. Silverman had initially found XMRV only in rare stromal cells adjacent to the tumor and not in epithelial cells. Not finding the virus in epithelial cells is problematic, Fan said. If a virus is implicated in cancer, the virus should theoretically exist in the tumor and surrounding cells. Only rarely does a virus “hit and run,” he said, meaning that it produces cancerous changes and then leaves no trace of its presence. This discrepancy might be due to Silverman and Singh using different antibodies to detect the virus. Silverman said he has found that the virus infects some prostate epithelial cells as well as stromal cells.

**Indirect Mechanisms?**

Researchers still have a long way to go to prove causality. “The pieces don’t all fit together,” Fan said. Unlike the human papillomavirus, XMRV lacks an oncogene, a gene that contributes to or causes a cell to become malignant. It is not known whether XMRV causes malignant changes by integrating into the DNA of human prostate cells near protooncogenes—normal genes that can become oncogenes. “Some retroviruses that lack oncogenes, such as most [murine leukemia viruses], can still cause tumors by integrating their genomes into host cell DNA in the vicinity of a protooncogene,” Fan said.

Because the virus was initially seen in nonmalignant cells only, it might be involved in an indirect mechanism of carcinogenesis, according to Silverman, Fan, and Chow. One indirect mechanism is chronic inflammation, which has been implicated in prostate cancer. Inflammation has been found in stromal cells near the tumors, and the virus may or may not be the cause, researchers say. Initial inflammation can set up a vicious cycle, in which even surrounding cells produce inflammatory cytokines and cause further inflammation that may contribute to malignant transformation. Chronic inflammation produced by certain bacteria and viruses is known to be a risk factor for some cancers.

“What is most interesting at the moment is this emerging picture for cancer in general that local inflammation can exacerbate or even initiate the oncogenesis process,” DeRisi said. “Since XMRV seems to be primarily in the surrounding fibroblasts, not the cancer cells themselves, perhaps they are contributing to local inflammation, which is in turn conducive to initiating full-blown tumor growth.”

One clue to XMRV’s indirect cancer-causing potential is that the virus, when integrating into human cells, seems to prefer sites near several genes that, if transformed, could contribute to tumorigenesis, Griffiths said.

Singh said her findings of the virus in malignant cells are more in line with classical models of retroviral oncogenesis: inactivation of a tumor suppressor gene or introduction of an oncogene. “This is different from the indirect model proposed by Silverman, in which cytokines and growth factors from infected stromal cells might promote proliferation of adjacent epithelial cells, leading them toward malignancy,” she said.

With so many unanswered questions, much work remains for the researchers; still unresolved, said Fan, are the frequency of XMRV infection in the human population, why the prostate is infected, and exactly how the virus fits into carcinogenesis. Goff is investigating what about the virus makes the prostate a hospitable environment and whether it, like MMTV, is hormone sensitive. Another question is whether XMRV is a true mouse virus or merely a human variant of a mouse virus.

Singh, Fan, and Goff are working to develop an animal model to help investigate the process of carcinogenesis and how XMRV replicates. “We plan to infect transgenic mice with XMRV and see if the mice develop prostate cancer,” said Singh, who also is an associate medical director of the University of Utah’s ARUP laboratories, a national reference lab.

With access to many thousands of tissue, serum, and other body fluid samples from ARUP Laboratories, Singh is also taking an epidemiological approach to determine how prevalent XMRV infection is. “We will look to see if women are infected with XMRV,” she said. “Is there any evidence of infection of cervical, ovarian, or breast tissue?”

Whether XMRV participates in the development of human prostate cancer, either familial or sporadic, remains uncertain, Griffiths said. Nevertheless, he added, “The discovery of the XMRV virus demonstrates that despite the controversy and skepticism associated with virus discovery, studies on new human retroviruses are still relevant today.”

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