CANDIDATE CANCER VIRUSES

Mouse Mammary Tumor Virus: New Tumor Virus or Just a Rumor Virus?

By Vicki Brower

When German microbiologist Harald zur Hausen, M.D., D.Sc., hypothesized in 1974 that human papillomavirus (HPV) played a central role in causing cervical cancer, few took him seriously. But in 1983 he discovered HPV-16, and a year later, HPV-18 in tumors of cervical cancer patients—findings that led eventually to the first preventive cancer vaccine and, for zur Hausen, last year’s Nobel prize for medicine.

Spurred by the clinical success of HPV research and the discovery of additional viruses implicated in cancer, scientists continue to hunt for other cancer-causing viruses. One leading contender, the mouse mammary tumor virus (MMTV), is a retrovirus that causes breast cancer in mice. Having found molecular evidence of its presence in human breast tumors, researchers are now investigating a possible causal link.

“Among ‘rumor viruses,’ perhaps the strongest case exists for MMTV in breast cancer,” said David Griffiths, Ph.D., of Scotland’s Moredun Research Institute in Penicuik.

Currently 15%-25% of cancers are virally linked, with six viruses considered cofactors in or causes of human cancer: HPV in cervical cancer, hepatitis B and C in liver cancer, Epstein–Barr virus in Burkitt lymphoma and nasopharyngeal cancer, human T-cell lymphotrophic virus type 1 in adult T-cell leukemia virus, and human herpesvirus 8 in Kaposi sarcoma. In addition to MMTV, other candidate viruses include simian virus 40 in mesothelioma, brain cancer, and lymphoma; cytomegalovirus in glioma; bovine leukemia virus in breast cancer and leukemia; Epstein–Barr virus in breast cancer; HPV in breast and prostate cancer; and xenotropic murine leukemia–related virus in prostate cancer.

Proving a causal link is difficult and time consuming (see JNCI News, 2004;96:256–7), and the MMTV hypothesis has gone in and out of favor over the last few decades. “In 1964, the NCI established its virus–cancer program, which focused on retroviruses after the discovery of [bovine leukemia virus] and other animal viruses, but 12–13 years of studies yielded nothing definitive,” said Gertrude Buehring, Ph.D., associate professor of virology at the University of California, Berkeley. “By the early 1980s, most everyone thought that the book was closed,” she said.

In addition, the discovery of human immunodeficiency virus in the mid-1980s diverted funding from MMTV research, according to Walter Gunzburg, Ph.D., professor of virology at the Veterinary University of Vienna. The NCI is not currently funding internal research of MMTV or other rumor viruses, but it does support external research on proven virus–cancer connections.

Evidence in Breast Cancer

An uptick in MMTV research occurred in the mid-1990s when researchers identified MMTV DNA sequences in human breast tumor samples. Beatriz Pogo, M.D., professor of medicine, hematology, medical oncology, and microbiology, and James Holland, M.D., professor of neoplastic diseases at New York City’s Mount Sinai Medical Center, examined normal and cancerous breast tissue DNA and found a 660-base pair segment of viral DNA sequence in 38% of breast cancer samples. The sequence was 98% similar to the MMTV envelope (env) gene. In addition to this molecular signature, they detected additional MMTV-like DNA sequences that represented a complete provirus; these sequences, known as human mammary tumor virus (HMTV), contain hormone-responsive elements, as does the mouse virus. They have been found, moreover, in breast cancer tissues and breast cancer cells, and they produce viral particles.
A second team, led by Polly Etkind, Ph.D., associate professor of oncology at the Montefiore Medical Center in New York, found HMTV sequences in breast tumor and lymphoma tissue in patients diagnosed with both malignancies, but not in normal breast tissue. Recently, Etkind identified HMTV sequences in the breast tumors of a husband, wife, and their adult daughter who lived together for decades in the same home.

Altogether, seven groups of researchers have found HMTV env gene sequences in human breast cancer tissue in 10 studies, Gunzburg said. Of the five controlled studies, only 1.7% of the normal breast tissue samples had HMTV sequences. Recently, Gunzburg’s group showed that MMTV can infect and rapidly spread in human breast cells and that it randomly integrates into human and mouse cells.

But to show causation, researchers must demonstrate how and where the virus enters human breast cells, that it survives and replicates, and that infection precedes disease. Exactly how it might cause disease in humans is unknown, although Susan Ross, Ph.D., professor of microbiology at the University of Pennsylvania in Philadelphia, has identified a segment of the env protein that drives malignant transformation of mammary epithelial cells in mice.

**Inflammation, Hormones, and Geography**

Many studies suggest that HMTV could influence the type and severity of breast cancer. James Lawson, M.D., professor emeritus of public health at Australia’s University of New South Wales in Sydney, demonstrated that 42% of invasive breast tumors studied had similar cellular characteristics to MMTV-induced mouse breast tumor specimens. Pogo’s team also showed that most of the tumors with HMTV sequences were invasive and correlated with overexpression of the laminin receptor, a marker for invasiveness and poor prognosis. Other research shows that HMTV is prevalent not only in more severe disease but also in gestational and inflammatory breast cancers, both of which are particularly virulent.

Gestational breast cancer is thought to be hormonally driven in humans. Although Pogo found that 30%–38% of sporadic breast cancer samples have HMTV env sequences, in gestational breast cancer samples the prevalence jumped to 62% in the U.S., supporting a possible hormonal link in humans. “We think that hormonal response elements present in MMTV play a role in promoting cancer cell growth in humans, as they do in mice,” Pogo said.

Another factor may be geography. In 2000, Canadian scientists proposed an environmental hypothesis regarding the geographical distribution of breast cancer. They theorized that humans acquire MMTV from one species of mice, *Mus domesticus*, the main carriers of the virus, and that areas in which these mice live coincide with locales with the highest rates of breast cancer in humans. *Mus domesticus* thrives in North Africa, South and North America, Australia, and Western Europe. Lawson theorizes that the increase in breast cancer rates that occurs among Asians who migrate to Western countries may be due not only to changes in diet and hormone levels, as many experts think, but also to increased exposure to MMTV.

In support of the geographic hypothesis, research from the early 1980s indicated that a virus resembling MMTV was strongly associated with breast cancer in Tunisian women, said Paul Levine, M.D., research professor of epidemiology and biostatistics at George Washington University in Washington, D.C. In 2004, he documented that 74% of Tunisian breast cancer patients had evidence of the virus, compared with 36% in the U.S., 38% in Italy, 42% in Australia, 31% in Argentina, 0.8% in Vietnam, and 1%–2% of healthy women.

Intriguingly, Tunisian women have higher rates of inflammatory breast cancer than those in other countries. Recently, Levine and colleagues showed that among U.S. women with inflammatory breast cancer, about 75% tested positive for the viral sequences. With funding from the Department of Defense, Levine has now established an inflammatory breast cancer registry and is investigating the disease’s association with suspected HMTV infection.

**Negative Findings**

Despite the surge of interest in HMTV, Ross and others in the field challenge the hypothesis that the mouse virus is connected to breast cancer in humans. Ross noted that if mice transmit the virus to humans, one would expect the genetic sequence to differ more than it does. “But
the HMTV sequences are not really different, only slightly, from MMTV if you compare all the strains of infectious and endogenous MMTVs,” she said. Scientists in Sweden and Japan have reported that they could not find the virus in human breast cancer cells, but Pogo and others question the sensitivity of their detection methods. Others ask whether contamination might account for evidence of HMTV in human breast cancer samples.

Ross stresses that no one yet has shown how the mouse virus enters human cells. Last year, she documented that the MMTV env protein binds to the mouse transferrin receptor to enter mouse cells and that it can bind to the human version of this receptor. However, it cannot enter human cells this way. “We tried to infect human cells with mouse virus and, after many tries, saw some very low levels of infection, which I would call ‘illegitimate.’ If you throw enough virus on cells, you might get infection through nonspecific means,” she said. “True infection requires binding to a cell receptor followed by viral–cell membrane fusion.”

Pogo counters that “retroviruses can infect cells without cognate receptors [by] using transactivation pathways [through another viral gene] … and retroviruses are known to cross species.”

The notion of a cancer virus jumping species is a sticking point for some, despite general acceptance that human T-cell lymphotropic virus, HIV, hantavirus, and the influenza viruses came from animals.

Researchers continue to work on solving this puzzle. Etkind is studying the mechanism by which the env protein transforms cells. Levine and colleagues are studying a possible inflammatory breast cancer–HMTV connection and working to develop a more sensitive antibody test that will help determine exposure to MMTV. And Holland is addressing the missing link of temporality—whether infection precedes disease. His team is examining large libraries of breast tissue samples from the Nurses’ Health Study, from which they will cull 300–400 samples from patients ultimately diagnosed with breast cancer, and 300 control samples, to determine whether HMTV infection precedes disease in women later diagnosed with breast cancer.

Gunzburg’s team is focusing on integration sites, and Pogo is investigating cells that are infected with HMTV, including lymphocytes, B cells, and T cells. She is also studying genes that are activated by the virus, as well as proteins that interact with the env gene.

If these studies do prove that MMTV causes breast cancer, the implications could be far reaching. “No one doubts HPV’s causal connection to cervical cancer,” Holland said. “If we can prove a causal connection between MMTV and breast cancer, then all tumors are open to the possibility of a viral connection.”

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