Connecting Viruses to Cancer:
How Research Moves From Association to Causation

In the past two decades, the study of viruses has contributed greatly to the understanding of cancer biology. But determining that a virus is a cause or a co-factor in cancer is another story, and it is slow, difficult work.

“As tools of discovery, viruses have turned out to be a Rosetta Stone for unlocking the mysteries of cell growth control,” said Janet Butel, Ph.D., professor of virology and chairman of the department of molecular virology and microbiology at the Baylor School of Medicine in Houston. Although about 15% of all human tumors worldwide are caused by viruses, there are only five viruses that have been incontrovertibly established as causing cancer, with numerous others under investigation.

Key to proving causation is the fulfillment of a set of criteria, some of which are difficult to prove. Although this area is active, with new research published monthly, it is also fraught with contention and bias, say some virologists.

“The first evidence of a possible causal link between a virus and a cancer is high—some say too high—and determining that a virus is a cause or a co-factor in cancer is another story, and it is slow, difficult work.”

To prove causation, there are four testable criteria:

1. The virus must be isolated from every case of the disease.
2. The virus must be recovered from a healthy susceptible host.
3. The specific disease must be reproducible in a healthy susceptible host; and the bacterium must be recoverable from the experimentally infected host.
4. There are different routes to proving causality; it takes time to accumulate a body of persuasive data, and there will always be a variety of studies collected with conflicting results,” said Butel, who is researching the connection between simian virus 40 (SV40) and human brain cancer. But finding virus–cancer connections is more than an academic exercise.

“The potential pay-offs of finding virus–human cancer links are new ways of diagnosis, treatment, and even prevention,” said Butel. If an infectious agent is isolated as a major cause, it may be possible to develop a vaccine to prevent disease. The hepatitis B vaccine has been used now for more than 15 years, and vaccines against human papillomavirus (HPV) are in clinical trials for the prevention of cervical cancer.

At present, accepted causal associations between viruses and human cancer are HPV and cervical cancer; human T-lymphotrophic virus type-1 (HTLV-1) and adult T-cell leukemia and lymphoma; hepatitis B and C and liver cancer, Epstein–Barr virus (EBV) and nasopharyngeal cancer, Burkitt’s and Hodgkin’s lymphomas; and some non-Hodgkin’s lymphomas; and human herpes virus 8 (HHV-8) and Kaposi’s sarcoma, according to the American Society of Clinical Oncology.

Other viruses under investigation include polyomaviruses in multiple cancers; the JC virus in brain and colon cancers; the BK virus in brain cancer; SV40 in non-Hodgkin’s lymphoma, mesothelioma, and cancers of the brain, pituitary gland, bone, and thyroid; hepatitis C and some non-Hodgkin’s lymphomas; the human equivalent of mouse mammary tumor virus (MMTV) and breast cancer; and HPV16 and ovarian cancer.

The first evidence of a possible causal link between a virus and cancer came in 1911, when a cell-free filtrate of tumor tissue from a sarcoma-afflicted chicken was injected into otherwise healthy chickens, which subsequently developed tumors that were discovered to be viral in origin. In the 1950s, mouse leukemia viruses were shown to be transmissible naturally from generation to generation in mice. Many viruses were found over the next 20 years that were tumorigenic, but attempts to isolate such viruses from humans was very difficult, Butel said. Although EBV was seen with electron microscopy in Burkitt’s lymphoma cells in 1964 and hepatitis B virus in 1970, many years passed before they were accepted as causes of human cancer, she added.

Improved laboratory techniques such as PCR and nucleic acid sequencing have made such research easier, but limitations of technology, such as the scarcity of animal models, still present challenges. And in spite of the possibility that viruses can be transmitted from animals to humans, many experts still question the likelihood of certain investigational viruses jumping from animals to infect humans and to cause cancer.

Guidelines developed to draw a link between pathogens and diseases such as SARS and AIDS were first formalized by German physician Robert Koch in 1890, and by Austin Bradford Hill in 1965. According to Koch, to determine whether a given bacterium is the cause of a particular disease, it must be present in every case of the disease; it must be isolated from the host with the disease and grown in pure culture; the specific disease must be reproducible in a healthy susceptible host; and the bacterium must be recoverable from the experimentally infected host.

However, viruses have characteristics that bacteria do not have. For example, over the years, scientists have discovered that viruses can “hit and run,” or infect a cell and leave little if any physical evidence of infection. They may remain latent for many years, or they may indirectly cause loss of cell cycle control. Often, viral replication does not occur in tumor cells, and the entire viral genome is not retained. These observations test the limitations of Koch’s postulates in relation to viruses.

Today, many scientists use the Bradford Hill criteria, which were developed to establish causation between a specific factor—environmental or otherwise—and a disease. The
criteria include strength of association, consistency (replication by other laboratories), specificity, temporality (whether exposure preceded disease), biologic dose gradient, plausibility (credible scientific mechanism), coherence with other evidence, experimental evidence, and analogy.

All of the seven criteria do not need to be fulfilled to show plausibility, however, said James Goedert, M.D., chief of the NCI’s Viral Epidemiology Branch, who has worked on HHV-8 and Kaposi’s sarcoma in AIDS. “There is no one set of information, no perfect checklist that represents definitive evidence for causality,” agreed Goedert’s colleague Rabkin, both of whom spoke recently at an ASCO-sponsored seminar on viruses and cancer. The Bradford Hill criteria are “sort of a mantra of epidemiologists,” Rabkin added. What is absolutely necessary, however, is temporality: The cause must be present in the organism prior to the outcome, Rabkin said. And the work must be replicated in numerous laboratories.

For proving the link between HPV and cervical cancer, gradient and plausibility were very strong: The former meant showing that there was an increase in risk with increasing sexual exposure, and the latter called for evidence that cervical dysplasia preceded cancer. For gastric cancer and Helicobacter pylori, there had been evidence linking infection with that bacterium to other diseases in the stomach, and finally, to precancerous changes, said Rabkin.

“Viruses are usually not complete carcinogens, and the known human cancer viruses display different roles in transformation,” Butel said. Many years may pass between initial infection and tumor appearance, and most infected individuals do not develop cancer, although, in some cases, immunocompromised people have higher risk of virus-associated cancer, she added. There is also a synergistic effect between some viruses and environmental co-factors.

Butel observed that, until two decades ago, there was no unifying theory for cancer’s origin, but scientists working with tumor viruses, chemical carcinogens, and other substances “were living in parallel universes, assuming their system was unique.” But in 1982, researchers discovered that transforming genes from human bladder and lung cancer were same as the ras genes identified on oncogenes in mouse sarcoma viruses. “Suddenly, it was apparent that the same cellular proto-oncogenes could be affected by viruses, chemicals or non-viral somatic mutations.” she said.

—Vicki Brower