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Review

The conundrum of causality in tumor virology: The cases of KSHV and MCV

Patrick S. Moore*, Yuan Chang

Cancer Virology Program, University of Pittsburgh Cancer Institute, 5117 Centre Avenue, Pittsburgh, PA 15213, United States

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ABSTRACT

Controversy has plagued tumor virology since the first tumor viruses were described over 100 years ago. Methods to establish cancer causation, such as Koch's postulates, work poorly or not at all for these viruses. Kaposi's sarcoma herpesvirus (KSHV/HHV8) and Merkel cell polyomavirus (MCV) were both found using nucleic acid identification methods but they represent opposite poles in the patterns for tumor virus epidemiology. KSHV is uncommon and has specific risk factors that contribute to infection and subsequent cancers. MCV and Merkel cell carcinoma (MCC), in contrast, is an example in which mutations to our normal viral flora contribute to cancer. Given the near-ubiquity of human MCV infection, establishing cancer causality relies on molecular evidence that does not fit comfortably within traditional infectious disease epidemiological models. These two viruses reveal some of the challenges and opportunities for inferring viral cancer causation in the age of molecular biology.

1. Introduction

Seven known human tumor viruses cause about 1 in every 6 cancers worldwide [1,2]. Beyond the large public health impact, this is remarkable because there are so few of these viruses: of the thousands of viruses causing infection, only a minute proportion have been established to cause cancer (Table 1) and even then most people infected with a cancer virus never develop tumors. This review focuses on the two most recently described tumor viruses, Kaposi's sarcoma herpesvirus (KSHV) and Merkel cell polyomavirus (MCV), which were discovered in 1994 and 2008, respectively. They reveal new opportunities, as well as new limits, for discovering infectious cancer causes in the age of molecular biology.

2. Causality, cancer and molecular virology

Controversies surround tumor viruses, largely on the fundamental question of whether or not they cause cancer. Causality itself is a topic that generates arguments not only among scientists but also among philosophers, statisticians, computer scientists, bar patrons and others. One tends to suppose that there exists well-defined criteria that must be met for an agent to be called a tumor virus. Either the agent meets these requirements or it does not. Instead, adjudicating causality is a normative process that no one person can successfully determine. Similar to a famous description for innovation, causality "only exists when the correctly credentialed hivemind agrees that it exists" [3]. But determining cancer virus causality is not an empty intellectual exercise because it has profound consequences that can be measured in lives prematurely lost when diagnostics, medicines and vaccines are not developed or employed.

EBV was discovered in 1964 [4], yet declared to be a legitimate human carcinogen only in 1997 by the International Agency for Cancer Research [5]. During these 32 years, ~3.7 million persons developed EBV-induced cancers (based on unadjusted 2008 estimates [1]). More recently the successes of human papillomavirus (HPV) and hepatitis B virus (HBV) control measures show that targeting the fundamental viral cause for a cancer can massively alter the burden of infectious cancers. The debate over AIDS and HIV provides an even more stark case for the practical importance of causal inference. Over 300,000 preventable HIV infections are estimated to have occurred in South Africa between 2000 and 2005 as a result of a government policy withholding distribution of antiretroviral prophylaxis for pregnant women on the basis that HIV is not the cause of AIDS [6]. This policy was supported by fringe science that did not take into account any modern sense of viral causality [7–9].

The reasons why viruses have until relatively recently been neglected as causes for cancer are complex [10]. Viral cancers—like all diseases—are multifactorial and only rare examples exist for a clear 1-to-1 correspondence between virus infection and neoplasia. Most persons who are exposed to a tumor virus never develop disease, although this should hardly be surprising since asymptomatic infection is a feature for almost all pathogens. Further, for every bona fide human cancer virus that has been found, there have been dozens of false leads and dead-ends that have littered the scientific literature with conflicting, confusing and contentious descriptions

* Corresponding author. Tel.: +1 412 623 7721.
E-mail addresses: psm9@pitt.edu (P.S. Moore), yc70@pitt.edu (Y. Chang).

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Table 1
Human tumor viruses.

Year	Virus	Abbreviation	Notable cancers
1964	Epstein–Barr virus	EBV	Burkitt's lymphoma, Nasopharyngeal carcinoma, Hodgkin disease, Gastric carcinoma
1965	Hepatitis B virus	HBV	Hepatocellular carcinoma
1980	Human T-lymphotropic virus-I	HTLV-1	Adult T cell leukemia
1983	High-risk human papillomavirus	HPV	Cervical cancer, Head and neck cancer
1989	Hepatitis C virus	HCV	Hepatocellular carcinoma
1994	Kaposi's sarcoma-associated herpesvirus	KSHV	Kaposi's sarcoma, Primary effusion lymphoma, Multicentric Castleman's disease
2008	Merkel cell polyomavirus	MCV	Merkel cell carcinoma

of virus-cancer links. Evidence that herpes simplex virus (HSV) 2 is the likely cause of cervical cancer led to a large body of evidence [11,12], the interpretation of which was clarified only after years of research following the discoveries of HPV type 16 and 18 by zur Hausen's group [11,13,14]. Since both HPV and HSV are sexually transmitted, confounding and overlapping epidemiologies for these two viruses is not surprising in retrospect. A more recent and remarkable example was discovery of a simple endogenous murine retrovirus, XMRV, which had cryptically jumped from the mouse genome into human prostate cancer cell lines during mouse xenograft passaging studies [15]. The virus was discovered over a decade later, long after the mouse passaging experiments had been forgotten, and therefore was reasonably suspected to be a novel human virus—though the discovering authors were appropriately cautious in ascribing any causative cancer etiology [16]. Each valid tumor virus requires years of confirmatory research to begin to unravel its association with cancers and so the failure to recognize true virus–cancer associations by the general public is understandable.

3. Classifying causation

For more detailed discussions of causal inference, see here [17–22]. Attempts to define causality date back at least to the era of Galileo Galilei (1564–1642) and (controversially) even have been ascribed to him [23]: an agent causes disease when it is both necessary and sufficient for the disease to occur. This has been called a complete causal effect [17].

Beyond the most simplistic examples, however, this is not a useful definition, especially not for infectious cancers. All infectious diseases emerge from a complex interplay of multiple factors (immunity, host genetics, age of infection, etc.), so that no infection alone is sufficient to cause cancer. Various causal factors (e.g., immunodeficiency and viral infection) may be overlapping and synergistic with each other so that the attributable risk from a viral infection may not be obvious [17]. Further, depending on how cancers are classified, not all types of a cancer would necessarily be caused by a single agent. A good example of this is hepatocellular carcinoma, which can arise after exposure to HBV, hepatitis C virus (HCV) or chemical mutagens [24]. And so, the necessary and sufficient definition becomes meaningless after even the most trivial scientific description of viral tumors.

Skepticism about causal inference reached a high point a century later when the empiricist David Hume (Fig. 1) outlined the difficulty, perhaps even impossibility, of describing causality through external criteria such as necessity and sufficiency (Fig. 2). Hume instead used a counterfactual argument to define causality: we can say object A causes object B when B follows A, and when A does not occur, then B does not occur [11,18,25].

4. Koch's postulates

This was hardly helpful to 19th Century microbiologists. To establish scientific rigor for the new field of microbiology, Robert Koch famously formulated a set of postulates for microbial causes of disease in a series of lectures to the International Congress in Berlin in 1890 [22]. These postulates have since tenaciously taken hold in microbiology because they are easy to remember and, at first glance, seem to be universally true. Koch's eponymous postulates were partially articulated by Koch's mentor, Jakob Henle in 1840 [26], and later expanded and codified by Koch so they are also often referred to as the Henle–Koch postulates [27]:

1. The parasite occurs in every case of the disease in question and under circumstances which can account for the pathological changes and clinical course of the disease.
2. It occurs in no other disease as a fortuitous and nonpathogenic parasite.
3. After being fully isolated from the body and repeatedly grown in pure culture, it can induce the disease anew.

The simplicity and clarity of this proposition is so persuasive that these postulates are usually included in the canon of a sound undergraduate science education. But, even Koch was aware of their limitations [8] during his disputes with Petenkoffer over whether *Vibrio cholerae*, which Koch had discovered [28], caused epidemic cholera disease (an argument leading Pettenkofer to swill pure cultures of the bacteria and then develop diarrhea but not classic cholera disease [29,30]). Even for diseases that have an intuitively obvious cause, rigorous application of Henle–Koch's postulates leave open possibilities for doubt. A skeptic could argue, for example, that *Neisseria meningitidis* is not the cause spinal meningitis since there are many infectious causes for meningitis and ~10% of healthy adults are asymptomatic *N. meningititdis* carriers. Even animal models for disease can be disputed as not fully recapitulating human spinal meningitis. Yet, detection of Gram-negative diplococci in cerebrospinal fluid leaves no reasonable room to doubt whether or not to give antibiotics to a symptomatic patient.

The principal problem with Henle–Koch's postulates is not so much that they are wrong but that they have little value since they are inapplicable to the majority of pathogens. When negative, the results say little about whether a candidate pathogen causes disease or not. For viruses, these issues become even more complex [31]. As obligate cellular parasites, viruses cannot be formally evaluated in pure culture. Under the most assiduous gradient isolation conditions, the purity of a virus culture can still be questioned. Viral cancers, which generally are non-permissive for viral replication [32], may have no virions to isolate and are therefore ineligible for the Henle–Koch rules. HIV denialists make use of these ambiguities, which in turn require complex—and ultimately unsatisfying—rebuttals by epidemiologists [33] trying to fulfill ersatz Henle–Koch's postulates. Ironically, Werner Henle, Jakob's grandson, and Werner's wife, Gertrude, contributed to establishing EBV as a cancer-causing virus by experimental lymphocyte immortalization studies [27] rather than by attempting to fulfill his grandfather's postulates. Henle–Koch's postulates are a brilliant example of precision in scientific thinking but they hold little practical value for 21st Century tumor virology since they cannot prove nor disprove most candidate tumor viruses to cause cancers. Rivers

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