Tumour-inducing viruses

Virus infections are an important factor in the global burden of human cancer. The discovery and mode of action of human tumour viruses is briefly reviewed together with the promise of prevention through vaccination.

Everyone knows that cancer is not catching in the sense that there is any risk of doctors or relatives developing cancer as a result of contact with a patient. Nonetheless, approximately 20% of the worldwide cancer burden has an infectious aetiology (De Martel et al, 2012). Various infectious agents, such as bacteria (e.g. *Helicobacter pylori* in stomach cancer) and helminths (carcinomas of the urinary bladder and gallbladder), are associated with malignancy, but approximately 1.5 million of the 2 million new cancer cases caused by infection each year develop as a result of virus infection.

When the *British Journal of Hospital Medicine* was founded 50 years ago, the Nobel Prize for Medicine & Physiology was awarded jointly to Dr Peyton Rous 'for his discovery of tumour-inducing viruses' and to Dr Charles Huggins 'for his discoveries concerning hormonal treatment of prostatic cancer' (Norrby, 2010). It was 55 years earlier that Rous had discovered the sarcoma virus in chickens that bears his name, representing the longest 'incubation period' between discovery and recognition in the annals of the Nobel Prizes (Weiss and Vogt, 2011). Given the coincidence of Rous's prize and the launch of this journal, it seems apt to review what has happened in the field of viral oncology during the ensuing half century.

Human oncogenic viruses

The first human virus linked to malignancy was discovered in paediatric cases of Burkitt's lymphoma 2 years before the journal's launch (Epstein et al, 1964). Today, eight groups of viruses are known to induce cancer (*Table 1*), the most recently discovered being Kaposi's sarcoma-associated virus (Chang et al, 1994) and Merkel cell polyoma virus (Feng et al, 2008). The viruses were identified through electron microscopy, isolation in culture, and molecular cloning methods.

All oncogenic viruses establish persistent infections, which typically occur many years before malignancy. The viral genome is usually present in the malignant cells and is sometimes integrated into chromosomal DNA. However, HIV and the RNA virus hepatitis C virus (Goossens and Hoshida, 2015) promote cancer indirectly. Oncogenic viruses belong to many virus families with different routes of transmission. Hepatitis B virus is frequently acquired perinatally or through subsequent exposure to blood. Human T-lymphotropic virus type 1 is transmitted vertically through infected cells in breast milk and via whole blood transfusion. Sexual transmission is common to HIV, hepatitis B virus, human papilloma virus and human T-lymphotropic virus type 1. Oncogenic

Table 1. Viruses involved in human cancer

Virus		Malignancy	Other disease
Epstein–Barr virus		Burkitt's lymphoma Diffuse large B-cell lymphoma Hodgkin's lymphoma Undifferentiated nasopharyngeal cancer Soft tissue sarcoma (leiomyosarcoma) Gastric adenocarcinoma	Infectious mononucleosis
Human papilloma viruses	Types 16, 18	Cervical carcinoma	
	Types 6, 11	Vulval carcinoma	Condyloma
	Type 5	Skin cancer in epidermodysplasia verruciformis	Warts
Hepatitis B virus		Hepatocellular carcinoma	Hepatitis, cirrhosis
Hepatitis C virus		Hepatocellular carcinoma	Hepatitis, cirrhosis
Polyoma virus		Merkel cell skin cancer	
Kaposi's sarcoma herpesvirus		Kaposi's sarcoma Primary effusion lymphoma Multicentric Castleman's disease	
Human T-lymphotropic virus type 1		Adult T-cell leukaemia	Autoimmune myelopathy
Human immunodeficiency virus		Viral tumours	Immune deficiency

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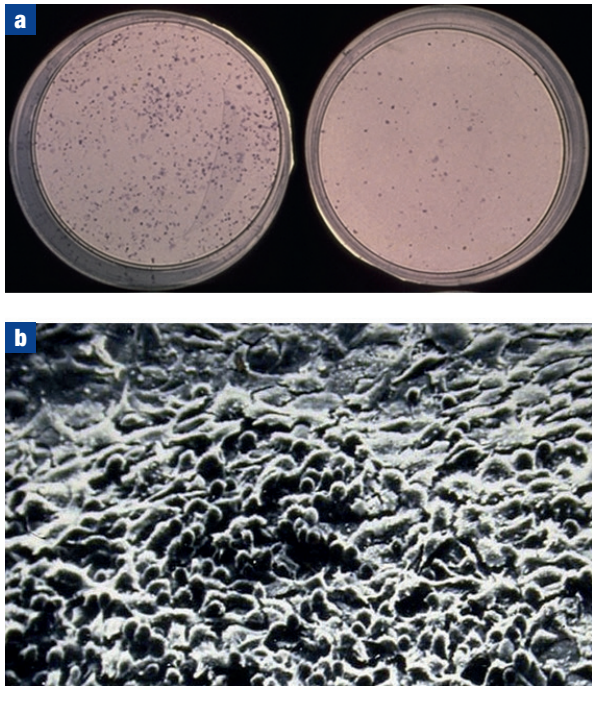
viruses do not appear to be transmitted either by the respiratory route or by arthropod vectors.

Cancer is a relatively rare outcome of virus infection, where cofactors play a role in oncogenesis (Schinzari et al 2015). For example, widespread infection by Epstein–Barr virus via saliva occurs in all human populations, but childhood Burkitt's lymphoma occurs only in areas of endemic malarial infection, and undifferentiated nasopharyngeal carcinoma occurs mainly in southern China. Human papilloma virus-5 and polyoma virus are ubiquitous skin infections yet the cancers they cause are extremely rare in immunocompetent individuals (Orth, 2006; Kassem et al, 2009). In hereditary epidermodysplasia verruciformis presenting with benign warts, ultraviolet radiation acts with cutaneous strains of human papilloma virus (usually human papilloma virus-5) to cause invasive skin cancer (Orth, 2006). In multifactorial diseases such as virally-induced cancers, what constitutes causality needs careful thought (Moore and Chang, 2014).

Figure 1. Malignant transformation of chick embryo fibroblasts in culture by Rous sarcoma virus. a. Virus stock diluted 1:100 (left panel) and 1:1000 (right panel) and incubated for 7 days; each stained dot represents a clone of transformed cells. **b.** Scanning electron micrograph of one clone of virus-transformed cells showing loss of contact inhibition of cell growth and invasion.



Viruses such as hepatitis B and hepatitis C viruses cause one specific type of cancer, hepatocellular cancer; the same tumour can develop in the absence of virus infection as a result of liver damage resulting from dietary mycotoxins or excessive alcohol consumption, although aflatoxin and hepatitis B virus act synergistically (Kirk et al, 2006). Human papilloma virus-16 and 18 are associated with squamous carcinomas of the uterine cervix (for which almost 100% are human papilloma virus-positive), the anus and approximately 40% of head and neck cancers (Ajila et al, 2015). Epstein–Barr virus is associated with a wide spectrum of lymphomas (Carbone et al, 2008), undifferentiated nasopharyngeal carcinoma (Hui at al, 2012), gastric carcinoma (Iizasa et al, 2012), possibly a subset of breast cancer (Richardson et al, 2015), and soft tissue sarcomas (Deyrup et al, 2006; Purgina et al, 2011) (*Table 1*).

Immune deficiency and iatrogenic immunosuppression are major risk factors for virus-induced malignancies (Chapman et al, 2013; Pierangeli et al, 2015). HIV can be considered as oncogenic because tumours such as cervical cancer in women, Kaposi's sarcoma and B-cell lymphomas are defining symptoms of AIDS (Boshoff and Weiss, 2002). AIDS-associated tumours can be regarded as 'opportunistic neoplasms' resulting from activation of latent oncogenic viruses.

Mechanisms of viral oncogenesis

It was the ability of polyoma viruses and retroviruses to transform cells in culture (*Figure 1*) that led to the discovery of oncogenes and tumour suppressor genes. Thus the study of tumour-inducing viruses provided fundamental insights into cancer in general. Most retroviruses do not carry oncogenes, but the DNA provirus integrates into chromosomal DNA and can activate adjacent cellular oncogenes like ras and myc to express positive signals for cell proliferation and invasion (Weiss and Vogt, 2011). This phenomenon is analogous to oncogene activation by chromosome translocation.

Tumour suppressor genes like p53 act as inhibitors. If their function is impaired either by mutation or by a viral protein blocking their cellular target then there is no regulation of cell proliferation. The p53 protein was first identified in cells transformed by the SV40 polyoma virus of macaques. We now know that many human tumour viruses, including human papilloma virus, polyoma virus and Kaposi's sarcoma herpesvirus, encode viral proteins that sequester p53 and Rb cellular proteins and redirect them to a ubiquinated degradation pathway (zur Hausen, 2009; Wendzicki et al, 2015).

Cell transformation by the human retrovirus human T-lymphotropic virus type 1 differs from the majority of animal retroviruses by encoding viral proteins, Tax and HBZ, which are essential for viral gene transcription (Matzuoka and Jeang, 2011). They also upregulate certain cellular genes such as the interleukin-2 receptor. Human T-lymphotropic virus type 1 immortalizes CD4+ T lymphocytes in culture,

as Epstein–Barr virus immortalizes B lymphocytes, but this is only one step in the complex pathway to malignancy.

Treatment

There is no general treatment of cancers that have a viral aetiology. Among the lymphoid malignancies, some respond well to radiotherapy or chemotherapy, such as Hodgkin's disease, whereas others seldom show remission, such as adult T-cell leukaemia. Thus human T-lymphotropic virus type 1 has a 50–60-year incubation period because the virus is usually acquired in infancy from infected cells in maternal milk, yet the mean survival once the malignancy becomes manifest is only ~10 months (Hermine, 2015).

Historically, research into tumour viruses helped the development of targeted therapies for specific malignancies (Stegmeier et al, 2010). For instance, imatinib interaction with the Bcr-abl tyrosine kinase inhibitor came from our understanding of viral leukaemias involving the abl oncogene in mice, and trastuzumab for HER-positive breast cancer was inspired by investigations of oncogenic viruses in chickens that encode related transmembrane tyrosine kinase receptors.

One would expect tumours that express 'foreign' viral antigens to be more responsive to immunotherapy and also to the patient's immune system mopping up surviving tumour cells after radiotherapy or chemotherapy. For example, human papilloma virus-positive squamous tumours of the head and neck show better survival odds than human papilloma virus-negative tumours (Ajila et al, 2015). For tumours in which viral proteins are required for the maintenance of the malignant state, these proteins are potential molecular targets. The EBNA 1 protein in tumours caused by Epstein–Barr virus is a candidate for this line of therapeutic development.

Prevention

Prevention can be approached through four different strategies:

1. Antiviral therapy before tumours develop
2. Early screening for viruses and virus-induced tumours
3. Screening for the virus in order to prevent transmission in blood transfusions or milk
4. Immunization.

Treatment of HIV and hepatitis C virus infection by antiviral drugs where cancer is a late consequence of chronic infection is playing a major role in cancer reduction. Early screening is exemplified by diagnostics using serology and cervical smear genomics for the multiple types of human papilloma virus (Heard et al, 2013; Gutierrez-Xicotencatl et al, 2016). Screening to prevent iatrogenic transmission via blood and blood products is routinely used for potentially oncogenic viruses such as hepatitis B virus, hepatitis C virus, HIV and human T-lymphotropic virus type 1 (Morrison et al, 2015). In Japan, where, human T-lymphotropic virus type 1 infection used to be endemic, the virus is being steadily eradicated through a policy of antenatal screening to prevent milk transmission.

> **"Certain viruses including parvoviruses and retroviruses can be exploited as vectors for immunization and for cancer gene therapy, either by restoring tumour suppressor functions or by enhancing immune responses through the local expression of antigens or cytokines"**

Prevention of cancer by immunization against infection by oncogenic viruses is likely to have a major impact on global cancer mortality. The hepatitis B virus vaccine is based on surface antigen and was the first vaccine to be based on the manufacture of a recombinant protein (Plymoth et al, 2009; Shouval et al, 2015). Two human papilloma virus vaccines protective against cervical cancer are based on empty virus-like particles using the recombinant VP1 protein (Astbury and Turner, 2009). The less frequent strains of oncogenic human papilloma virus are being added to form multivalent human papilloma virus vaccines. Although intensive research is being undertaken on vaccines for HIV and hepatitis C virus, there are obstacles to successful design because the viral antigens are extraordinarily variable.

Exploiting viruses as cancer therapeutic agents

In malignant cells, the tumour virus is not fully replicating, so activation of the lytic cycle can lead to cytotoxicity and tumour reduction (Hui et al, 2012). Viruses that are not themselves oncogenic can also help the fight against cancer. Some cytopathic viruses can be manipulated to replicate selectively in proliferating cells and destroy them (Breitbach et al, 2016; Yuan et al, 2016). A variant of oncolysis is 'xenogenization' in virus infection which makes the tumour cell look more foreign and can also boost the immune response to non-infected cancer cells (Russell and Peng, 2007; Ferguson et al, 2012). Moreover, certain viruses including parvoviruses and retroviruses can be exploited as vectors for immunization and for cancer gene therapy, either by restoring tumour suppressor functions or by enhancing immune responses through the local expression of antigens or cytokines (Amer, 2014). Viral vectors can also deliver genes for enzymes that convert inert prodrugs into active chemotherapeutic agents (Zhang et al, 2015).

Conclusions and future prospects

There are likely to be exciting developments in the relatively near future on targetting viral oncogenic proteins more precisely and on harnessing viruses for cancer treatment. On the maxim that prevention is better than cure, I would hope that before the *British Journal of Hospital Medicine* reaches its centenary, we will have eradicated most viral tumours through vaccines. Oncologists may then view tumour-inducing viruses as historical curiosities or perhaps not be aware of them at all! **BJHM**

KEY POINTS

- Approximately 15% of all human cancer has a viral aetiology, and vaccines hold great promise to reduce that burden.
- Diverse types of virus can cause cancer, often through common molecular pathways. Oncogenic viruses have given us much insight into cancer more generally, e.g. the discovery of oncogenes and tumour suppressor genes.
- Cancer can be viewed as a 'side effect' of persistent viral infection, and often requires co-factors.
- Immune deficiencies, such as those caused by HIV infection or taking immunosuppressive drugs, markedly increase the incidence of virus-induced cancers.
- Viruses can also be exploited to treat cancer.

Figure 1 *is reproduced with permission from RA Weiss, PhD Thesis, University of London, 1969.*

Conflict of interest: none.

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