Cancers are the result of a disruption of the normal restraints on cellular proliferation. It is apparent that the number of ways in which such disruption can occur is strictly limited and there may be as few as forty cellular genes in which mutation or some other disruption of their expression leads to unrestrained cell growth.

There are two classes of these genes in which altered expression can lead to loss of growth control:

- Those genes that are stimulatory for growth and which cause cancer when hyperactive. Mutations in these genes will be dominant. These genes are called oncogenes.
- Those genes that inhibit cell growth and which cause cancer when they are turned off. Mutations in these genes will be recessive. These are the anti-oncogenes or tumor-suppressor genes.

Viruses are involved in cancers because they can either carry a copy of one of these genes or can alter expression of the cell's copy of one of these genes. These are the oncogenic virus (otherwise known as oncoviruses or tumor viruses).
To understand the discovery of cellular proto-oncogenes
To learn how cellular oncogenes may cause cancer in the absence of a virus
To learn understand how these discoveries led to the discovery of anti-oncogenes
To understand how the discovery of anti-oncogenes showed how DNA viruses may cause cancer

CLASSES OF TUMOR VIRUSES

There are two classes of tumor viruses:
- DNA tumor viruses
- RNA tumor viruses, the latter also being referred to as RETROVIRUSES.

We shall see that these two classes have very different ways of reproducing themselves but they often have one aspect of their life cycle in common: the ability to integrate their own genome into that of the host cell. Such integration is not, however, a pre-requisite for tumor formation.

TRANSFORMATION AND ONCOGENES

If a virus takes up residence in a cell and alters the properties of that cell, the cell is said to be transformed. Transformation by a virus is the change in the biological properties of a cell that results from the regulation of the cell by viral genes and that confer on the infected cells certain properties of neoplasia.

Transformation often includes loss of growth control, anchorage-independent growth, ability to invade extracellular matrix, dedifferentiation and immortalization. In carcinomas, many epithelial cells undergo an epithelial-mesenchymal transformation. Transformed cells often exhibit chromosomal aberrations and the changes seen in transformation often, but not always, result from the integration of the viral genome into the host cell's chromosomes.

The region of the viral genome (DNA in DNA tumor-viruses or RNA in RNA-tumor viruses) that can cause a tumor is called an oncogene. This foreign gene can be carried into a cell by the virus and cause the host cell to take on new properties.

The discovery of viral oncogenes in retroviruses led to the finding that they are not unique to viruses and homologous genes (called proto-oncogenes) are found in all cells. Indeed, it is likely that the virus picked up a cellular gene during its evolution and this gene has subsequently become altered. Normally, the cellular proto-oncogenes are not expressed in a quiescent cell since they are involved in growth (which is not occurring in most cells of the body) and development; or they are expressed under strict control by the cell. However, they may become aberrantly expressed when the cell is infected by tumor viruses that do not themselves carry a viral oncogene. We shall see later how this happens but it is clear that a virus may cause cancer in two ways: It may carry an oncogene into a cell or it may activate a cellular proto-oncogene.

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The discovery of cellular oncogenes opened the way to the elucidation of mechanisms by which non-virally induced cancers may be caused. We shall investigate what the protein products of the viral and cellular oncogenes do in the infected cell and in cells in which cellular proto-oncogenes are expressed. We shall see that their functions strongly suggest mechanisms by which cells may be transformed to a neoplastic phenotype. The discovery of cellular oncogenes led to the discovery of another class of cellular genes, the tumor repressor (suppressor) genes or anti-oncogenes.

Initially, the involvement of viral and cellular oncogenes in tumors caused by retroviruses was much more apparent than the involvement of the DNA tumor virus oncogenes but the discovery of tumor repressor genes (as a result of our knowledge of how retroviruses cause cancer) led to the elucidation of the mode of action of DNA virus oncogenes.
It should be noted that while retroviruses have been instrumental in elucidation of the mechanisms of oncogenesis, most human cancers are probably not the result of a retroviral infection although retroviruses are important in cancers in some animals. It is becoming much more apparent that many human tumors may result from infection by DNA tumor viruses.

**DNA TUMOR VIRUSES**

DNA tumor virus have a DNA genome that is transcribed into RNA which is translated into protein (figure 1). They have two life-styles:

- In permissive cells, all parts of the viral genome are expressed. This leads to viral replication, cell lysis and cell death
- In cells that are non-permissive for replication, viral DNA is usually, but not always, integrated into the cell chromosomes at random sites. Only part of the viral genome is expressed. This is the early, control functions (e.g. T antigens) of the virus. Viral structural proteins are not made and no progeny virus is released.

**DNA TUMOR VIRUSES INVOLVED IN HUMAN CANCERS**

The first DNA tumor viruses to be discovered were rabbit fibroma virus and Shope papilloma virus, both discovered by Richard Shope in the 1930s. Papillomas are benign growths, such as warts, of epithelial cells. They were discovered by making a filtered extract of a tumor from a wild rabbit and injecting the filtrate into another rabbit in which a benign papilloma grew. However, when the filtrate was injected into a domestic rabbit, the result was a carcinoma, that is a malignant growth. A seminal observation was that it was no longer possible to isolate infectious virus from the malignant growth. This was because the virus had become integrated into the chromosomes of the malignant cells.

**SMALL DNA TUMOR VIRUSES**

**FAMILY: PAPILLOMAVIRIDAE**

**PAPILLOMA VIRUSES**

The Papillomaviridae were formerly classified with the Polyomaviridae within the family Papovaviridae (so named for Pa: papilloma; Po: polyoma; Va: vacuolating). This term is no longer used, the papillomas and polyomas now being considered separate families.

The papillomaviridae are small non-enveloped icosahedral DNA viruses (figure 2). The major capsid protein, L1, is present as 72 pentamers (capsomers). This protein is all that is required to form the icosahedral capsid which occurs by self assembly. Each pentamer is associated with one molecule of another minor capsid protein, either L2 or L3. Papilloma viruses have a genome size about 8 kilobases and the DNA is complexed with histone proteins encoded by the host cell.
These viruses cause warts (figure 3A) and also human and animal cancers. Warts are usually benign but can convert to malignant carcinomas. This occurs in patients with epidermodysplasia verruciformis (figure 3B).

Epidermodysplasia verruciformis is also known as Lewandowsky-Lutz dysplasia or Lutz-Lewandowsky epidermodysplasia verruciformis and is very rare. It is an autosomal recessive mutation that leads to abnormal, uncontrolled papilloma virus replication. This results in the growth of scaly macules and papules on many parts of the body but especially on the hands and feet. Epidermodysplasia verruciformis, which is associated with a high risk of skin carcinoma, is typically associated with HPV types 5 and 8 (but other types may also be involved). These infect most people (up to 80% of the population) and are usually asymptomatic.

Papilloma viruses are also found associated with human penile, uterine, cervical and anal carcinomas and are very likely to be their cause; moreover, genital warts can convert to carcinomas.

Squamous cell carcinomas of larynx, esophagus and lung appear very like cervical carcinoma histologically and these may also involve papilloma viruses. Recently, a strong causal link between certain oral-pharyngeal cancers and HPV16 has been demonstrated.

There are more than 100 types of human papilloma viruses but, clearly, not all are associated with cancers; however, papillomas may cause 16% of female cancers worldwide and 10% of all cancers.

Vulvar, penile and cervical cancers are associated with type 16 and type 18 papilloma viruses (and others) but the most common genital human papilloma viruses (HPV) are types 6 and 11. As might be expected if they are indeed the causes of certain cancers, types 16 and 18 cause transformation of human keratinocytes. In a German study, it was shown that 1 in 30 HPV type16-infected women will develop malignant disease while 1 in 500 infected people develop penile or vulvar cancer. Since not all infected persons develop a cancer, there are probably co-factors in stimulating the disease. Such co-factors have been identified in alimentary tract carcinomas in cattle where a diet containing bracken fern is associated with the disease. People with HIV infection or AIDS are at increased risk of HPV-associated cancers as are patients with other forms of immunosuppression.

The fact that a virus is usually found in association with a disease (often, in the case of tumors, the presence of a copy of the viral genome in the neoplastic cells) does not prove that the virus caused the cancer. The association could be causal rather than causal. Nevertheless, in many instances the epidemiological data are very strong and, in the case of human cervical cancer, the efficacy of the anti-HPV vaccine makes the contention that HPV does cause cervical cancer very compelling.

<table>
<thead>
<tr>
<th>HPV vaccine</th>
<th>appears highly effective against cervical cancer</th>
<th>HPV may cause oral cancer</th>
<th>HPV types that may cause human cancer</th>
<th>HPV vaccines: Gardasil and Cervarix</th>
</tr>
</thead>
</table>

Figure 3B

Epidermodysplasia verruciformis: This widespread, markedly pruritic, erythematous eruption was eventually found to be caused by human papillomavirus infection. International Association of Physicians in AIDS Care

Epidermodysplasia verruciformis: Hyperkeratotic warty lesions on dorsal aspect of hands

Epidermodysplasia verruciformis: Histopathological view: Koilicyttes and
moderate dysplasia in the epidermis (H&E x100)

From: Reza Mahmoud Robati MD, Afshan Macefat MD, Marjan Saeedi MD, Mohammad Rahmati-Roodsari MD, Zahra Asadi-Kani MD
Dermatology Online Journal 15 (4): 8, 2009 (used under Creative Commons license)

Verrucous carcinoma. The epithelium shows surface maturation, parakeratosis, and hyperkeratosis. There is little or no cellular atypia. The stroma shows a mild chronic inflammatory infiltrate.

The Johns Hopkins Autopsy Resource (JHAR) Image Archive.

Figure 4A
Transmission electron micrograph of polyomavirus SV40 Dr. Erskine Palmer CDC

Figure 4B
Human polyomaviruses and associated diseases. The organs to which each human polyomavirus has tropism and causes disease.

doi:10.1371/journal.ppat.1003206.g001
From: The Rapidly Expanding Family of Human Polyomaviruses: Recent Developments in Understanding Their Life Cycle and Role in Human Pathology. Martyn K. White, Jennifer Gordon and Kamel Khalili. PLOS Pathogens. Used under Creative Commons License

WEB RESOURCES
Cutaneous manifestations of human papilloma virus
Epidermodysplasia verruciformis E-medicine

FAMILY: POLYOMAVIRIDAE

POLYOMA VIRUSES

The polyomaviridae (figure 4A) are small non-enveloped icosahedral DNA viruses (figure 2). The major capsid protein, VP1, is present as 72 pentamers. Each pentamer is associated with one molecule of another minor capsid protein, either VP2 or VP3. They have a genome of about 5 kilobases. Each particle is about 40–50 nanometers across.

Until recently, there was only one genus of polyoma viruses. However, more have been discovered and in 2010, the single genus was split into three:

- Orthopoxvirus This contains the classic mammalian polyomaviruses (e.g., JCPyV, BKPyV, SV40, mouse polyomavirus, etc.);
- Wukipoxvirus This contains the recently discovered human polyomaviruses including Karolinska Institute polyomavirus (KIPyV) and the Washington University polyomavirus (WUPyV);
- Avipoxvirus. This contains the avian polyomaviruses

Many polyoma viruses have been associated with human disease (figure 4b).

Mouse (Murine) Polyoma virus
Polyoma virus was so named because it causes a wide range of tumors in a number of animal species at many different sites. It was originally isolated from AK mice and is fully permissive for replication in mouse cells. It causes leukemias in mice and hamsters.

Simian virus 40
SV40 virus was initially discovered in the rhesus monkey kidney cells that were used to make inactivated Salk polio vaccine virus. It was found that when the inactivated polio virus made in these cells was added to African Green Money Kidney cells, the vaccine gave a cytopathic effect indicative of the presence of a live virus that had not been killed by the formalin used to inactivate the vaccine virus. SV40 replicates in rhesus monkey kidney cells but has no cytopathic effect on them. Many early recipients of the Salk polio vaccine received contaminating SV40 since anti-SV40 antibodies (against a protein called the large tumor antigen (T-antigen)) could be detected in their blood. No elevated incidence of cancer has been found in these people.

Although SV40 is a monkey virus that has no apparent effect on the host animal, it causes sarcomas when injected into juvenile hamsters. The hamster tumor cells produce no infective virus.

Human polyoma viruses
The first two human polyoma isolates, known as BK and JC were discovered in 1971. Neither came from a tumor. BK came from the urine of a kidney transplant patient and JC came from the brain of a Hodgkin’s lymphoma patient who progressed to progressive multifocal leukoencephalopathy (PML); however, they cause tumors when injected into animals. 70 to 80% of the human population is seropositive for JC. This virus is known to be the cause of PML (see slow viral diseases), a disease associated with immunosuppression. In 1979, the rate of occurrence of this disease was 1.5 per 10
million population. It has become much more common because of AIDS and is seen in 5% of AIDS patients. BK virus is an important cause of nephropathy and graft failure in immuno-suppressed renal transplant recipients and almost everyone in western countries has anti-BK virus antibodies by the age of 10. Recently, BK viral DNA has been associated with human prostate cancer.

Three other human polyoma viruses have recently been described: KI, WU and Merkel cell polyoma virus. The latter virus causes a rare skin cancer (Merkel cell carcinoma, see box below).

<table>
<thead>
<tr>
<th>BK virus and human prostate cancer</th>
<th>A polyoma virus may cause a rare skin cancer</th>
<th>Polyoma viruses and human disease</th>
</tr>
</thead>
</table>

Polyoma viruses are usually lytic (cause lysis) and when transformation occurs, it is because the transforming virus is defective. After integration into host DNA, only early functions are transcribed into mRNA and expressed as a protein product. These are the tumor antigens. Because the expression of the genes for tumor antigens is essential for transformation of the cells, they may be classified as oncogenes.

DEFINITION OF AN ONCOGENE: AN ONCOGENE IS A GENE THAT CODES FOR A PROTEIN THAT POTENTIALLY CAN TRANSFORM A NORMAL CELL INTO A MALIGNANT CELL. IT MAY BE TRANSMITTED BY A VIRUS IN WHICH CASE WE REFER TO IT AS A VIRAL ONCOGENE.

FAMILY: ADENOVIRIDAE

ADENOVIRUSES

These viruses (figure 5) are somewhat larger than polyoma and papilloma viruses with a genome size of about 35 kilobases. They were originally isolated from human tonsils and adenoids, are highly oncogenic in animals and only a portion of the virus is integrated into the host genome. This portion codes several T antigens that carry out early functions. Tumor-bearing animals make antibodies against the T antigens.

No humans cancers have been unequivocally associated with adenoviruses.

TUMOR ANTIGENS ARE ONCOGENES

Tumors caused by papilloma virus, adenovirus or polyoma virus contain viral DNA but do not produce infectious virus. The presence of the virus, however, elicits the formation of antibodies against the tumor antigens. In the case of adenoviruses, only part of the viral genome is found in the host cell chromosomes whereas SV40 may integrate part or all of its genome. Whether or not the whole SV40 genome is integrated, only a part of the genome is transcribed into mRNA and protein and this is the region that encodes the early functions of the virus replication cycle.
Many DNA viruses have early and late functions. Early functions are the result of the expression of proteins that prime the cell for virus production and are involved in viral DNA replication. These proteins are expressed before genome replication and do not usually end up in the mature virus particle. Late functions are the results of the expression of viral structural proteins that combine to form the mature virus. They are expressed during and after the process of DNA replication. Since early functions are involved in the replication of the viral genome, it is not surprising that they can also alter the replication of host cell DNA.

SV40 expresses two such proteins, the T antigens (large T and small T antigen). The large T antigen acts as a cis-regulatory element at the level of viral DNA replication by binding to the origin of replication and stimulating transcription. It can also bind to and modulate the activity of host cell DNA polymerase alpha.

As we shall see later, DNA replication in the cell is controlled by suppressor proteins (the best studied of which are the retinoblastoma (Rb) and p53 suppressor proteins). SV40 large T antigen can bind directly to these proteins and inactivate them, thereby inducing the cell to go from G0 to S phase. Because polyoma viruses have a small genome, they rely on many cell functions for DNA replication and it is important that the virus causes the cell to enter S phase because it creates a suitable environment for viral DNA replication.

Thus, SV40 Large T antigen:

- is necessary for transformation of a cell to the cancerous state
- stimulates the host cell to replicate its DNA
- is found mostly in the nucleus (to which it is directed by its nuclear localization signal) but a small amount goes to the cell surface (where it is a tumor-specific transplantation antigen)
- binds to cellular DNA
- binds to p53 protein (see below)

A second T antigen (small T antigen) interacts with a family of cellular phosphatases (called pp2A) which results in the failure of certain cellular proteins to be phosphorylated, thereby relieving cell cycle arrest.

In mouse polyoma virus, there is a middle T antigen which can also act as an oncogene.

Similarly, in adenovirus-induced tumors, only a part of the viral genome becomes integrated and again it is the early region genes. This region codes for the E1A and E1B proteins. In papilloma virus-induced tumor, again, two early genes, E6 and E7, are expressed.

Thus, papilloma, polyoma and adenoviruses seem to cause cell transformation in a similar manner: the integration of early function genes into the chromosome and the expression of these DNA synthesis-controlling genes without the production of viral structural proteins. As we shall see later, all three virus types induce cell proliferation by interacting with tumor suppressor genes.

Two important points that should be emphasized about T antigens of DNA tumor viruses as oncogenes:

- They are true viral genes. There are no cellular homologues in the uninfected cell
- They are necessary in lytic infections because they participate in the control of viral and cellular DNA transcription

*These properties should be contrasted with retroviral oncogenes to be discussed later*
FAMILY: HERPESVIRIDAE

HERPESVIRUSES

Herpesviruses (figure 6) are much larger than the DNA viruses described above and have a genome size of 100 to 200 kilobases. Because of their large size, a lot remains to be discovered concerning the way in which these viruses transform cells.

There is considerable circumstantial evidence that implicates these large enveloped viruses in human cancers and they are highly tumorigenic in animals. The herpes virus genome integrates into the host cell at specific sites and may cause chromosomal breakage or other damage (see below). Herpesviruses are often co-carcinogens. They may have a hit and run mechanism of oncogenesis, perhaps by expressing proteins early in infection that lead to chromosomal breakage or other damage.

Herpesviruses have over 100 genes. When these viruses infect cells which are non-permissive for virus production but which are transformed, only a subset (about 9) of viral genes are expressed. These genes code for nuclear antigens or membrane proteins. Not all nine transformation-associated genes are expressed in all herpes-transformed cells.

Epstein-Barr virus (Human herpes virus 4)

EBV (figure 7A) is the herpes virus that is most strongly associated with cancer. It infects primarily lymphocytes and epithelial cells. In lymphocytes, the infection is usually non-productive, while virus is shed (productive infection) from infected epithelial cells.

EBV is causally associated with:

- Burkitt's lymphoma (figure 7B) in the tropics (figure 7C), where it is more common in malaria-endemic regions
- Nasopharyngeal cancer, particularly in China and SE Asia, where certain diets may act as co-carcinogens
- B cell lymphomas in immune suppressed individuals (such as in organ transplantation or HIV)
- Hodgkin's lymphoma in which it has been detected in a high percentage of cases (about 40% of affected patients)
- X-linked lymphoproliferative Disease (Duncan's syndrome)

EBV can cause lymphoma in Marmosets and transform human B lymphocytes in vitro.

EBV also causes infectious mononucleosis, otherwise known as glandular fever (figure 7D). This is a self-resolving infection of B-lymphocytes which proliferate benignly. Often infection goes unnoticed (it is sub-clinical) and about half of the population in western countries has been infected by the time they reach 20 years of age. Why this virus causes a benign disease in some populations but malignant disease in others is unknown.
Why is Burkitt’s lymphoma restricted to certain parts of the world?

X-linked lymphoproliferative disease and EBV

Figure 7A
Epstein-Barr Virus

Figure 7B
Burkitt’s Lymphoma caused by Epstein-Barr Virus

Figure 7C
Distribution of Burkitt’s lymphoma

Figure 7D
Peripheral blood smears from a healthy individual (A) and a patient with infectious mononucleosis caused by Epstein-Barr virus (EBV) (B). Both smears are stained with Giemsa stain © Gloria J. Delisle and Lewis Tomalty Queen’s University Kingston, Ontario, Canada and The MicrobeLibrary

Human Herpes Virus 8 (HHV-8, Kaposi’s Sarcoma Herpes Virus)

HHV-8 infects lymphocytes and epithelial/endothelial cells and is the causative agent of Kaposi’s sarcoma. It has also been associated with hematologic malignancies, including primary effusion lymphoma, multicentric Castleman’s (also Castelman’s) disease (MCD), MCD-related immunoblastic/plasmablastic lymphoma and various atypical lymphoproliferative disorders.

EBV and HHV-8 have been found to be associated with oral lesions and neoplasms in HIV-infected patients. Among these diseases is oral hairy leukoplakia (OHL, figure 7E) which is benign and causes white thickenings on the tongue epithelium in which these viruses proliferate.

Human cytomegalovirus (Human Herpes Virus 5)

Castelman’s disease
This herpes virus (figure 7F) is frequently associated with Kaposi's sarcoma but this disease is now thought probably to be caused by human herpes virus 8.

For more on herpes viruses and the diseases that they cause, go to Virology Chapter 11 Herpes Viruses

FAMILY: HEPADNAVIRIDAE

HEPATITIS B VIRUS

Hepatitis B virus (figure 9) is very different from the other DNA tumor viruses. Indeed, even though it is a DNA virus, it is much more similar to the oncornaviruses (RNA tumor viruses) in its mode of replication. The DNA is transcribed into RNA not only for the manufacture of viral proteins but for genome replication. Genomic RNA is transcribed back into genomic DNA. This is called reverse transcription. The latter is not typical of most DNA tumor viruses but reverse transcription is a very important factor in the life cycles of RNA-tumor viruses. See below.

For more information on the molecular biology of hepatitis B virus and the diseases it causes, go to chapter 18 and chapter 19, part 2.

Hepatitis B is a vast public health problem and hepatocellular carcinoma (HCC) (figure 8), which is one of world's most common cancers, may well be caused by HBV. There is a very strong correlation between HBsAg (hepatitis B virus surface antigen) chronic carriers and the incidence of HCC. In Taiwan, it has been shown that HBsAg carriers have a risk of HCC that is 217 times that of a non-carrier. 51% of deaths of HBsAg carriers are caused by liver cirrhosis or HCC compared to 2% of the general population.
hepatocellular carcinoma and oncogenesis in hepatocellular carcinoma

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