MICROBIOLOGY COURSE MATERIAL

Semester - III

DR. PRIYADARSHINI MALLICK

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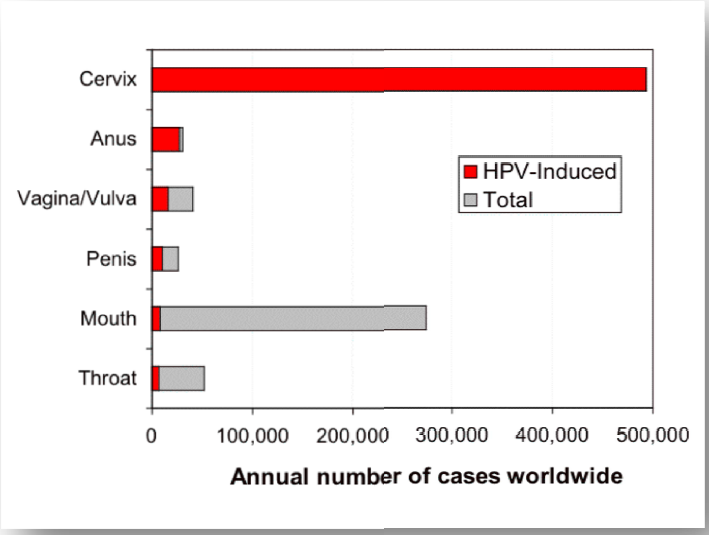
CC5: UNIT 4: PART B: CANCER VIRUSES

B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)
SEMESTER – III
CC5: UNIT – 4: PART- B
CANCER VIRUSES

❖ Cancer Viruses

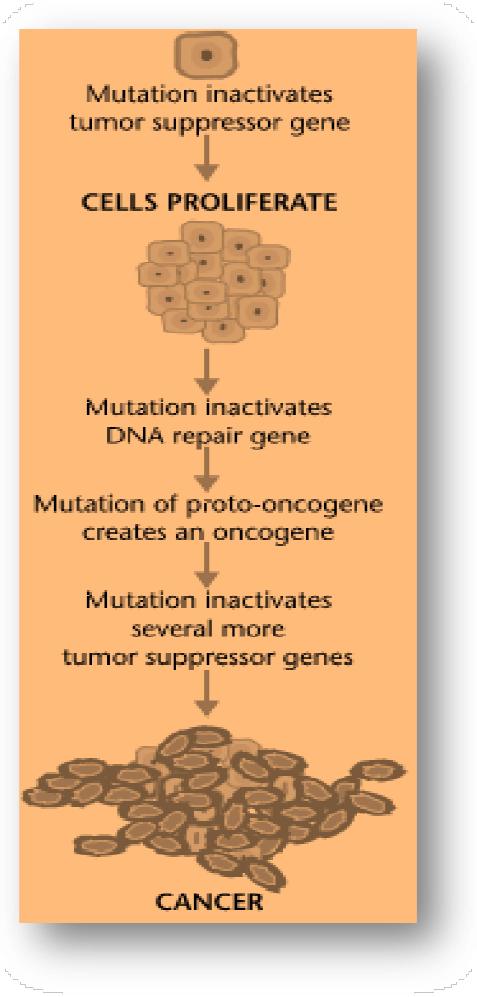
Viruses can cause cancer by transforming a normal cell into a malignant cell. Worldwide, cancer viruses are estimated to cause 15-20% of all cancers in humans. Most viral infections, however, do not lead to tumor formation; several factors influence the progression from viral infection to cancer development. These factors include host's genetic makeup, mutation occurrence, exposure to cancer causing agents, and immune impairment.



Viruses typically initiate cancer development by suppressing the host's immune system, causing inflammation over a long period of time, or by altering host genes. Cancer cells have characteristics that differ from normal cells, such as acquiring the ability to grow uncontrollably. This can result from having control of their own

growth signals, losing sensitivity to anti-growth signals, and losing the ability to undergo apoptosis, or programmed cell death.

Cancer cells don't experience biological ageing, and maintain their ability to undergo cell division and growth. Transformation occurs when a virus infects and genetically alters a cell. The infected cell is regulated by the viral genes and has the ability to undergo abnormal new growth. Scientists have been able to discern some commonality among viruses that cause tumors. The tumor viruses or oncoviruses change cells by integrating their genetic material with the host cell's DNA. Unlike the integration seen in prophages, this is a permanent insertion; the genetic material is never removed.



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 is 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).

❖ Mutations Leading to Increased Cell Division:

Cancer is caused by a series of mutations. Viral infections contribute to the process through genetic alteration. The insertion mechanism can differ depending on whether the nucleic acid in the virus is DNA or RNA. In DNA viruses, the genetic material can be directly inserted into the host's DNA. RNA viruses must first transcribe RNA to DNA and then insert the genetic material into the host cell's DNA. There are two classes of cancer viruses: DNA and RNA viruses. Several viruses have been linked to certain types of cancer in humans. These viruses have varying ways of reproduction and represent several different virus families.

❖ DNA Oncogenic Viruses

DNA Oncogenic Viruses include the following:

- Human Papilloma Virus is strongly linked to the development of cervical cancer among other types of cancers.
- The Epstein-Barr Virus has been linked to Burkitt's Lymphoma. This virus infects B cells of the immune system and epithelial cells.
- The Hepatitis B Virus has been linked to liver cancer in people with chronic infections.
- Human Papilloma Viruses have been linked to cervical cancer. They also cause warts and benign papillomas.
- Human Herpes Virus-8 has been linked to the development of Kaposi sarcoma. Kaposi sarcoma causes patches of abnormal tissue to develop in various area of the body including under the skin, in the lining of the mouth, nose, and throat or in other organs.

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, a malignant growth. A seminal observation was that it was no longer possible to isolate infectious virus from the malignant growth because the virus had become integrated into the chromosomes of the malignant cells.

❖ RNA Oncogenic Viruses

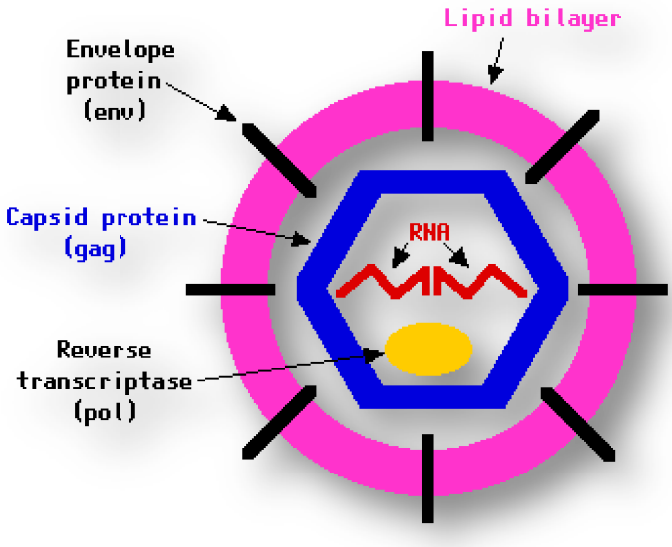
These viruses have varying ways of reproduction and represent several different virus families. Specifically, RNA viruses have RNA as their genetic material and can be either single-stranded RNA (ssRNA) or double-stranded (dsRNA). RNA viruses are classified based on the Baltimore classification system and do not take into account viruses with DNA intermediates in their life cycle. Viruses which contain RNA for their genetic material but do include DNA intermediates in their life cycle are called "retroviruses". There are numerous RNA oncogenic viruses that have been linked to various cancer types. These various oncogenic viruses include:

1) Human T Lymphotrophic Virus Type 1 (HTLV-I), a retrovirus, has been linked to T-cell leukemia.
2) Hepatitis viruses includes Hepatitis B and Hepatitis C which have been linked to hepatocellular carcinoma. The Hepatitis C Virus has been linked to liver cancer in people with chronic infections.
3) Human Papilloma Viruses (HPV) have been linked to cancer of the cervix, anus, penis, vagina/vulva, and some cancers of the head and neck.
4) Kaposi's Sarcoma associated Herpes Virus (HHV-8) has been linked to Kaposi's sarcoma and primary effusion lymphoma.
5) Epstein-Barr Virus (EBV) has been linked to Burkitt's Lymphoma, Hodgkin's Lymphoma, post-transplantation lymphoproliferative disease, and nasopharyngeal carcinoma.

❖ RNA Retroviruses

Retroviruses are different from DNA tumor viruses in that their genome is RNA, but they are similar to many DNA tumor viruses in that the genome is integrated into host genome. Since RNA makes up the genome of the mature virus particle, it must

be copied to DNA prior to integration into the host cell chromosome. This lifestyle goes against the central dogma of molecular biology in which the DNA is copied into RNA. The outer envelope comes from the host cell plasma membrane. Coat proteins (surface antigens) are encoded by env (envelope) gene and are glycosylated. One primary gene product is made, but this is cleaved so that there are more than one surface glycoprotein in the mature virus (cleavage is by host enzyme in the Golgi apparatus). The primary protein (before cleavage) is made on ribosomes attached to the endoplasmic reticulum and is a transmembrane (type 1) protein. Inside the membrane is an icosahedral capsid containing proteins encoded by the gag gene (group-specific AntiGen). Gag-encoded proteins also coat the genomic RNA. Again, there is one primary gene product. This is cleaved by a virally-encoded protease (from the pol gene). There are two molecules of genomic RNA per virus particle with a 5′ cap and a 3′ poly A sequence. Thus, the virus is diploid. The RNA is plus sense (same sense as mRNA). About 10 copies of reverse transcriptase are present within the mature virus, these are encoded by the pol gene. Pol gene codes for several functions (again, as with gag and env, a polyprotein is made that is then cut up).



Diagrammatic view of a RNA Retrovirus

❖ Classes of tumor viruses

There are two classes of tumor viruses:

- DNA tumor viruses
- RNA tumor viruses, also referred to as RETROVIRUSES.

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 the extracellular matrix, de-differentiation 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. A virus may cause cancer in two ways: It may carry an oncogene into a cell or it may activate a cellular proto-oncogene.

The discovery of cellular oncogenes opened the way to the elucidation of mechanisms by which *non-virally induced cancers* may be caused. 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.

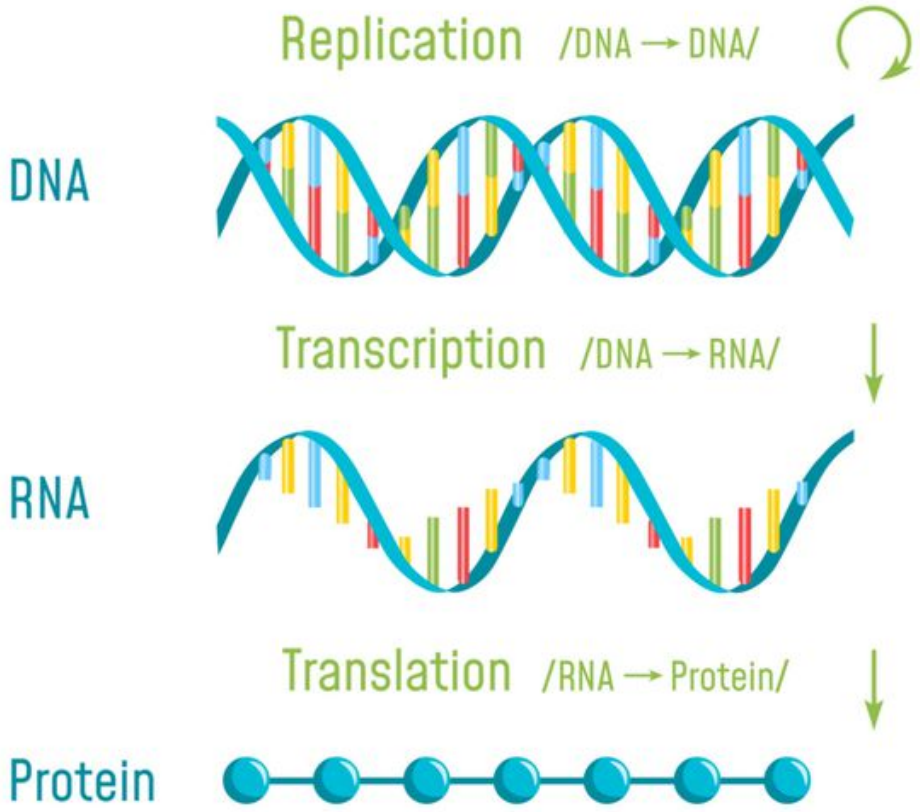
❖ DNA tumor viruses

DNA tumor viruses have a DNA genome that is transcribed into RNA which is translated into protein. 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.



❖ SMALL DNA TUMOR VIRUSES

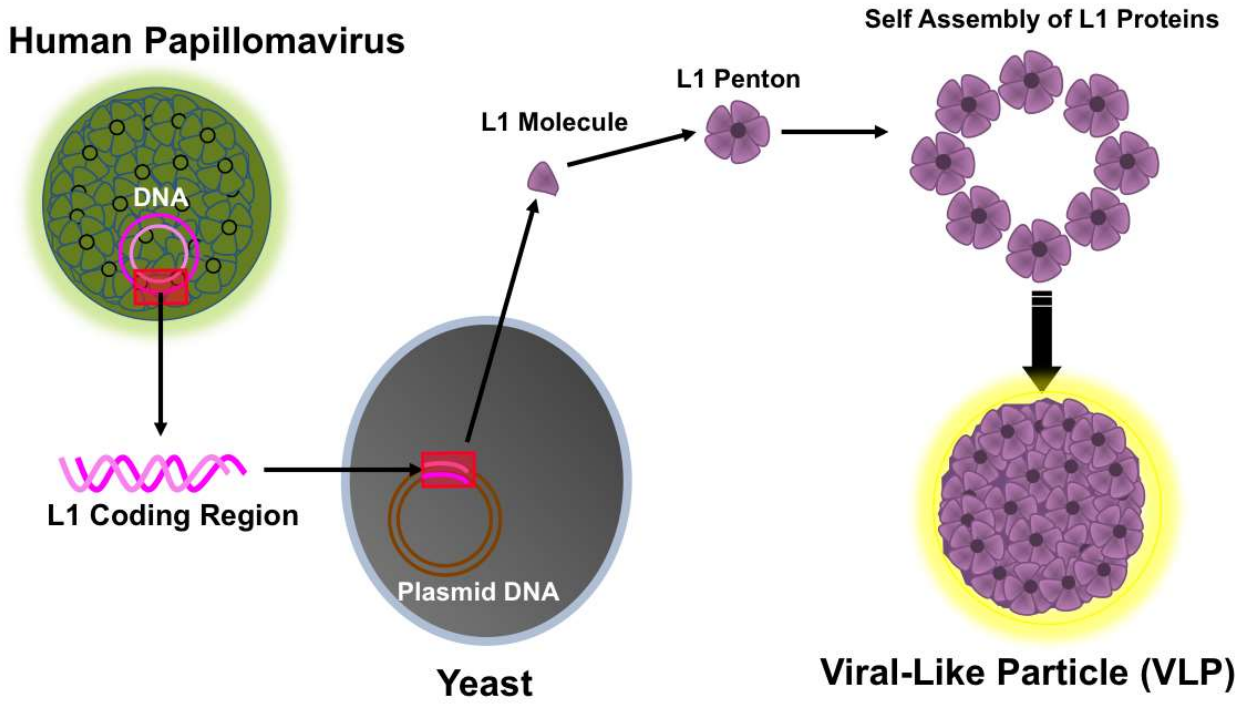
➢ FAMILY: PAPILLOMAVIRIDAE - PAPILLOMA VIRUSES

- 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. 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) and also human and animal cancers. Warts are usually benign but can convert to malignant carcinomas. This occurs in patients with *Epidermodysplasia verruciformis*.



Diagrammatic View of Human Papilloma Virus Infection

- *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 casual. 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.

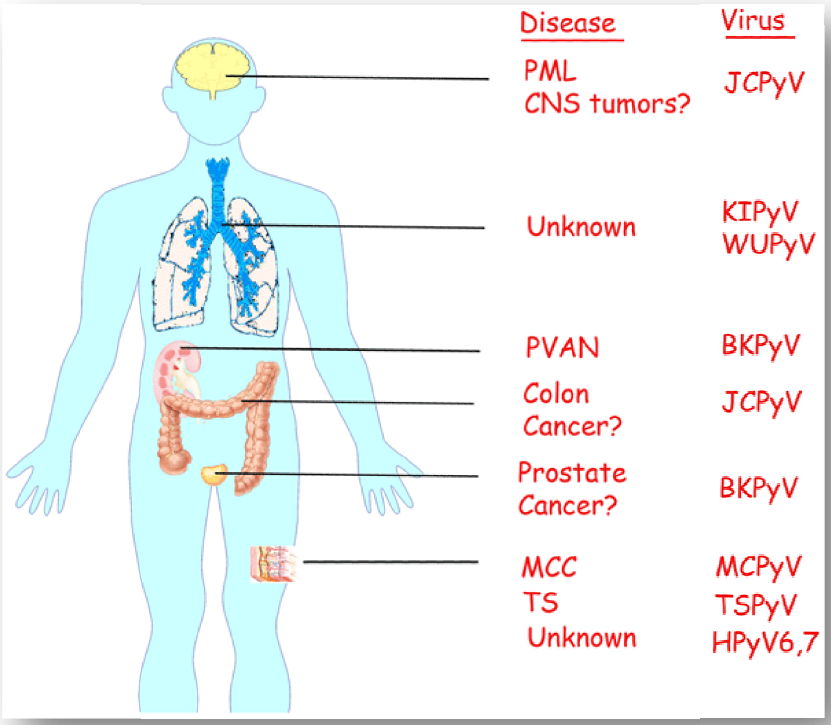
➢ FAMILY: POLYOMAVIRIDAE - POLYOMA VIRUSES

The polyomaviridae are small non-enveloped icosahedral DNA viruses. 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:

- **Orthopolyomavirus:** This contains the classic mammalian polyomaviruses (e.g., JCPyV, BKPyV, SV40, mouse polyomavirus, etc.);

- **Wukipolyomavirus**: This contains the recently discovered human polyomaviruses including Karolinska Institute Polyomavirus (KIPyV) and the Washington University Polyomavirus (WUPyV);

- **Avipolyomavirus:** This contains the avian polyomaviruses. Many polyoma viruses have been associated with human disease.



❖ 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

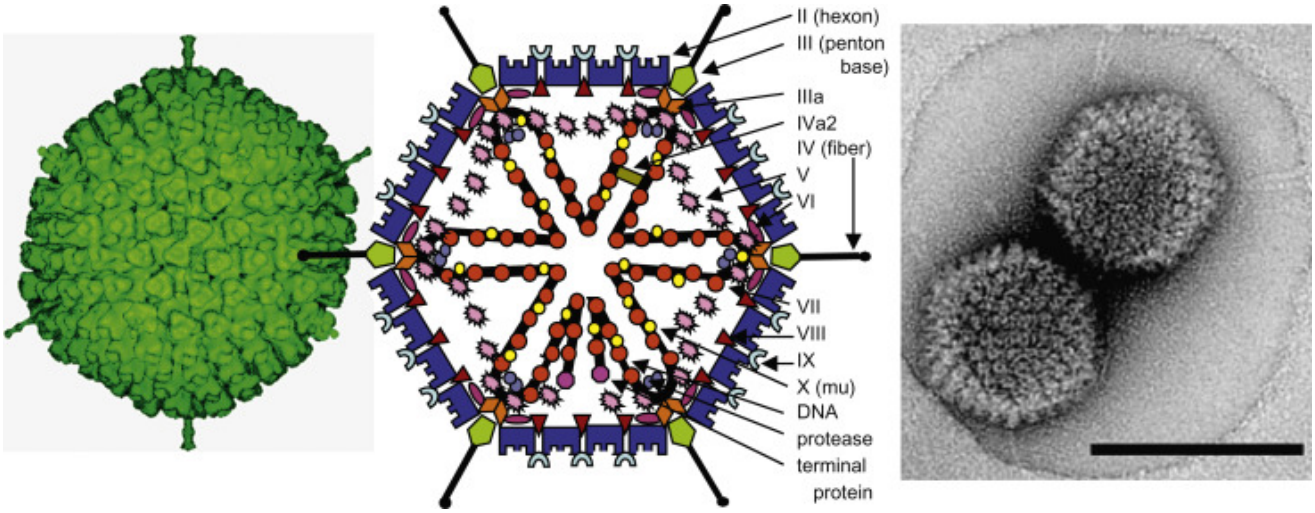
The first two human polyoma isolates, known as BK and JC were discovered in 1971. Neither came from a tumor. BK came from the urine of a kidney transplant patient and JC came from the brain of a Hodgkin's Lymphoma patient who progressed to Progressive Multifocal Leukoencephalopathy (PML); however, they 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, a disease associated with immunosuppression. In 1979, the rate of occurrence of this disease was 1.5 per 10 million populations. 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). 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.

❖ FAMILY: ADENOVIRIDAE - ADENOVIRUSES

These viruses (figure) 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 human cancers have been unequivocally associated with adenoviruses.



Structure of Adenoviridae

❖ 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.

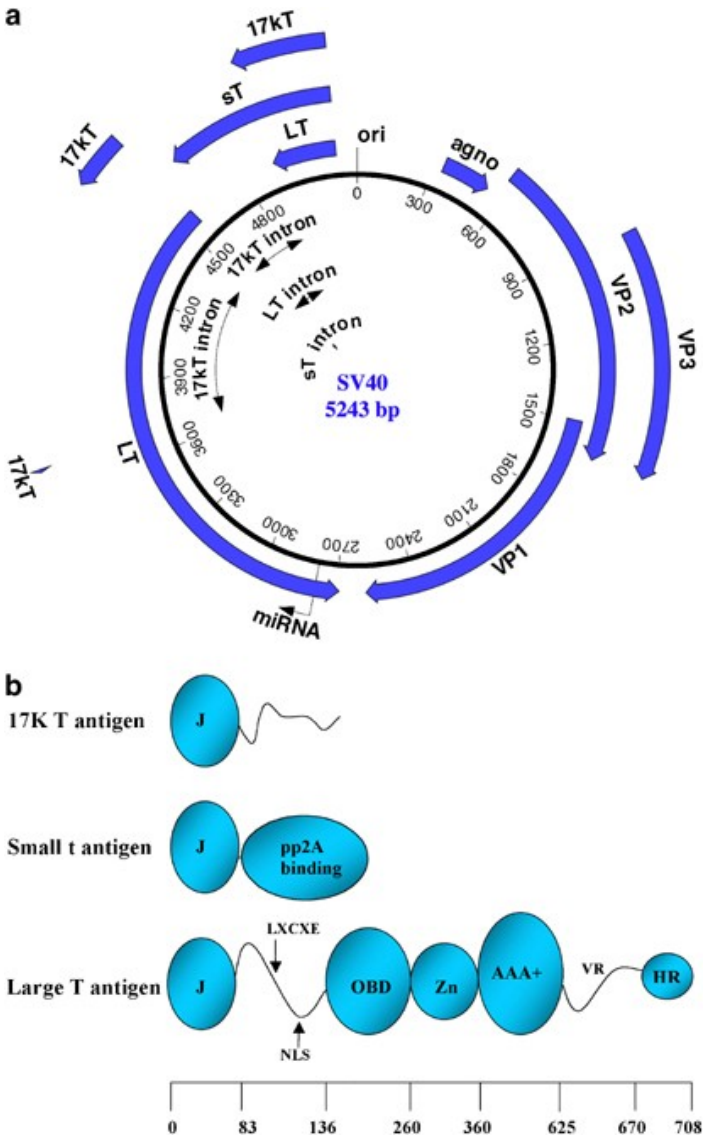
Many DNA viruses have early and late functions. Early functions are the result of the expression of proteins that prime the cell for virus production and are involved in viral DNA replication. These proteins are expressed before genome replication and do not usually end up in the mature virus particle. 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.

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 G_o 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)



SV40 large T antigen targets multiple cellular pathways to elicit cellular transformation

- binds to cellular DNA
- binds to p53 protein

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:

1. the integration of early function genes into the chromosome and
2. the expression of these DNA synthesis controlling genes without the production of viral structural proteins.

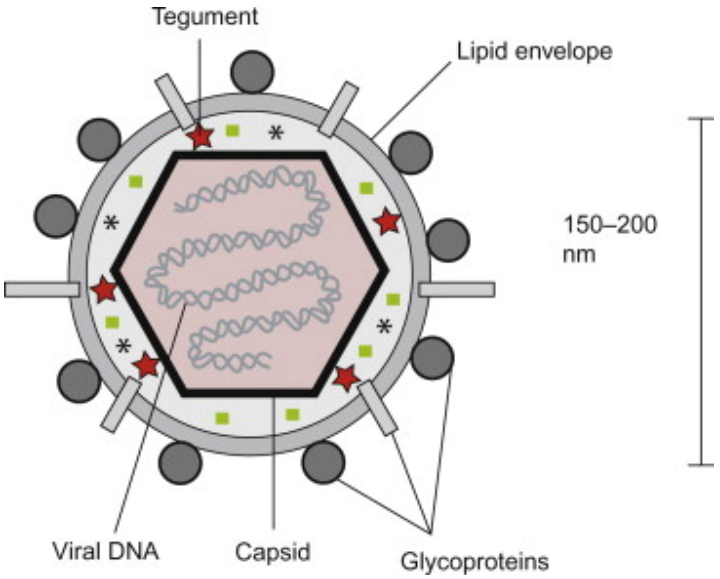
All three virus types induce cell proliferation by interacting with tumor suppressor genes. Two important points that should be emphasized about T antigens of DNA tumor viruses as oncogenes:

- 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.

❖ COMPLEX TUMOR VIRUSES

➢ FAMILY: HERPESVIRIDAE - HERPESVIRUSES

Herpes Viruses (figure) 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. Herpes Viruses 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. Herpes Viruses 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 of nuclear antigens or membrane proteins. Not all nine transformation-associated genes are expressed in all herpes-transformed cells.



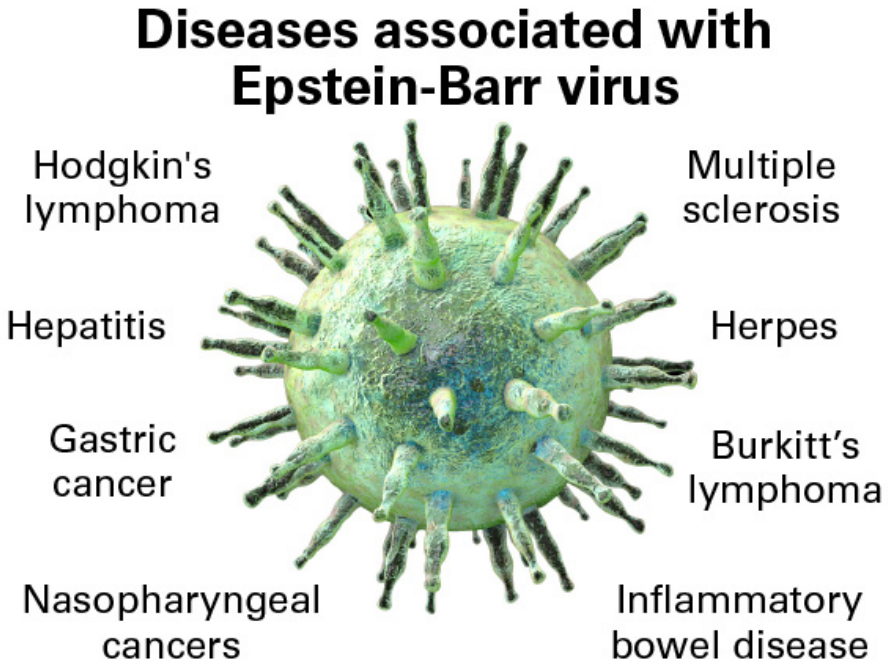
Structure of Herpes Virus

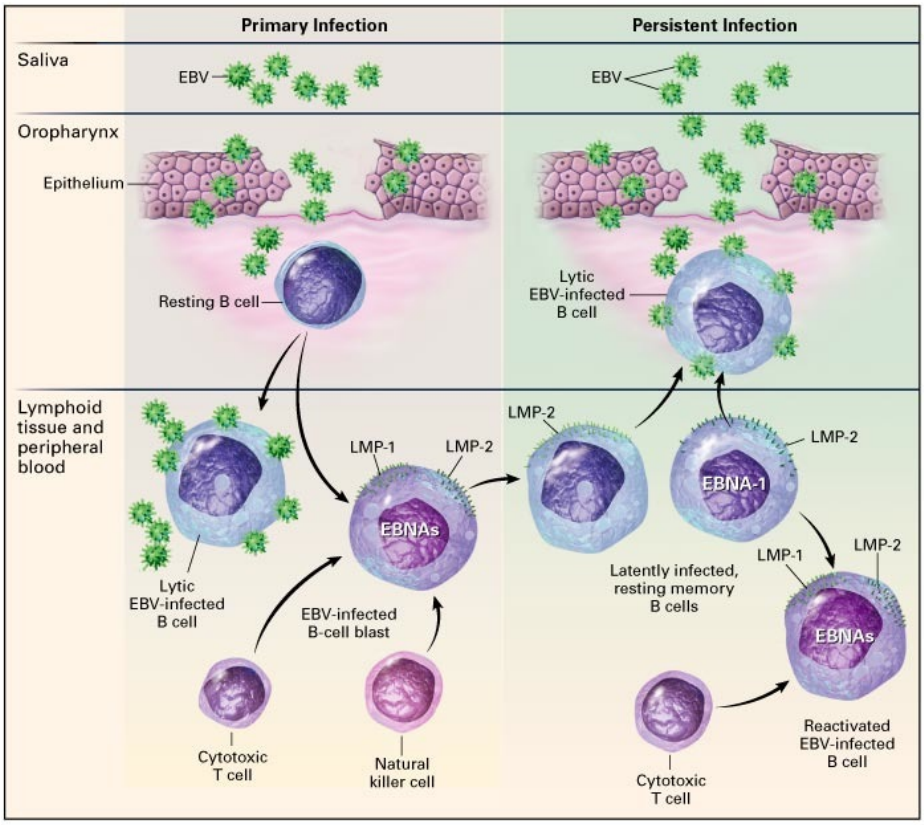
❖ Epstein-Barr virus (Human Herpes Virus 4)

EBV (figure) 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 in the tropics 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 i*n vitro*.

EBV also causes infectious mononucleosis, otherwise known as glandular fever. 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.





Epstein-Barr Virus Infection

❖ 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) which is benign and causes white thickenings on the tongue epithelium in which these viruses proliferate.

➢ FAMILY: HEPADNAVIRIDAE - HEPATITIS B VIRUS

Hepatitis B virus (figure) 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. Hepatitis B is a vast public health problem and hepatocellular carcinoma (HCC) (figure), 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.

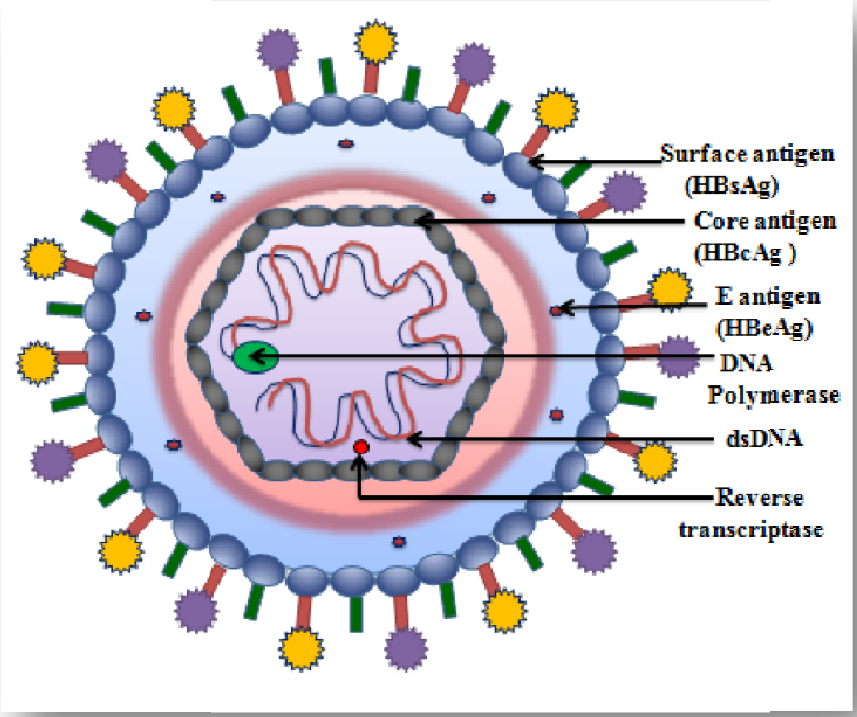
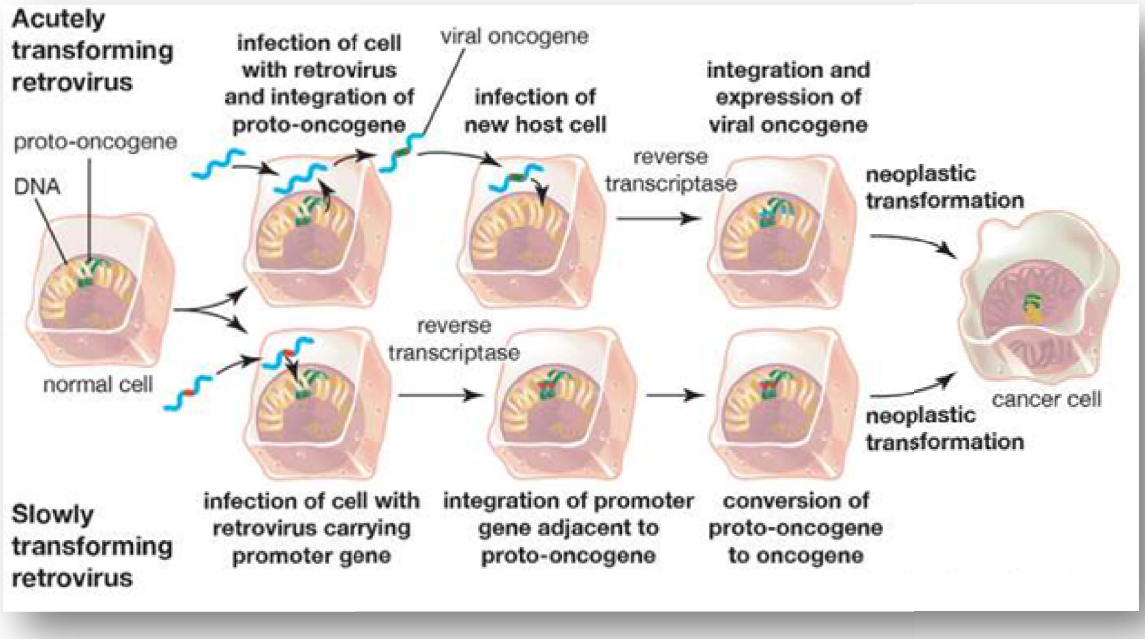


Diagram of Hepatitis B Virus

❖ Oncogene

Oncogene is the genetic material that carries the ability to induce cancer. An oncogene is a sequence of deoxyribonucleic acid (DNA) that has been altered or mutated from its original form, the proto-oncogene. Operating as a positive growth regulator, the proto-oncogene is involved in promoting the differentiation and proliferation of normal cells. A variety of proto-oncogenes are involved in different crucial steps of cell growth, and a change in the proto-oncogene's sequence or in the amount of protein it produces can interfere with its normal role in cellular regulation. Uncontrolled cell growth, or neoplastic transformation, ultimately resulting in the formation of a cancerous tumour.



❖ Cancer-causing retroviruses

Retroviral insertion can convert a proto-oncogene, integral to the control of cell division, into an oncogene, the agent responsible for transforming a healthy cell into a cancer cell. An acutely transforming retrovirus (shown at top), which produces tumours within weeks of infection, incorporates genetic material from a host cell into its own genome upon infection, forming a viral oncogene. When the viral oncogene infects another cell, an enzyme called reverse transcriptase copies the single-stranded genetic material into double-stranded DNA, which is then integrated into the cellular genome. A slowly transforming retrovirus (shown at bottom), which requires months to elicit tumour growth, does not disrupt cellular function through the insertion of a viral oncogene. Rather, it carries a promoter gene that is integrated into the cellular genome of the host cell next to or within a proto-oncogene, allowing conversion of the proto-oncogene to an oncogene.

Oncogenes first were discovered in certain retroviruses (viruses composed of RNA instead of DNA and that contain reverse transcriptase) and were identified as

cancer-causing agents in many animals. In the mid-1970s, the American microbiologists John Michael Bishop and Harold Varmus tested the theory that healthy body cells contain dormant viral oncogenes that, when triggered, cause cancer. They showed that oncogenes are actually derived from normal genes (proto-oncogenes) present in the body cells of their host.

With DNA sequences similar, but not identical, to their viral equivalents, proto-oncogenes occur naturally within the genomes of a wide variety of vertebrate species, including humans, but do not cause cellular transformation. Although a useful function of the proto-oncogene was not initially apparent, and it was believed to be "silent" or not expressed until being "switched on" to cause uncontrolled growth, its importance in cell regulation was soon identified.

The similarity between viral and cellular oncogenes can be explained by the life strategy of the retrovirus. The virus inserts itself into the genome of the host cell in order to replicate and then removes itself to infect other cells, sometimes capturing a portion of the host cell's genome along with its own. If a proto-oncogene has been integrated into a virus' own genetic material, its proper regulation may not be possible given the limited genetic repertoire of the retrovirus and it is transformed into an oncogene.

The term *proto-oncogene* was coined to distinguish the normal gene from its altered form. The resulting nomenclature is somewhat misleading. *Onco-*, from the Greek *onkos*, meaning "bulk," or "mass," refers to the tumour-causing ability of the oncogene, which is apt, but the term *proto-oncogene* stresses the potential that the gene has to become a malignant force, rather than its integral role as a regulator of cell activity.

Oncogenes, as with all other genes, are often designated by abbreviations (e.g., *MYC* and *RAS*). The origin or location of the gene is indicated by the prefix of "v-

" for virus or "c-" for cell or chromosome; additional prefixes, suffixes, and superscripts provide further delineation. More than 70 human oncogenes have been identified. Breast cancer has been linked to the c-*ERBB2* (HER2) oncogene and lung cancer to the c-*MYC* oncogene. Oncogenes arising in members of the *RAS* gene family are found in 20 percent of all human cancers, including lung, colon, and pancreatic.

In humans, proto-oncogenes can be transformed into oncogenes in three ways, all of which result in a loss of or reduction in cell regulation.

1. An alteration of a single nucleotide base pair, called a point mutation, can arise spontaneously or as a result of environmental influences such as chemical carcinogens or ultraviolet radiation. This seemingly minor event can lead to the production of an altered protein that cannot be properly regulated. Point mutations are responsible for converting certain *RAS* proto-oncogenes to oncogenes.
2. A second method of oncogenesis occurs by the process of translocation, in which a segment of the chromosome breaks off and attaches to another chromosome. If the dislocated chromosome contains a proto-oncogene, it may be removed from its regulatory controls and be continuously produced. The excess production of protein molecules disrupts the cellular process normally under their control, thereby destabilizing the delicate balance of the mechanisms of cell growth. Many leukemias and lymphomas are caused by translocations of proto-oncogenes.
3. The third method of transformation involves amplification in the number of copies of the proto-oncogene, which also can result in overproduction of the protein and its concomitant effects. Amplified proto-oncogenes have been found in tumours from patients with breast cancer and neuroblastoma (a tumour of the sympathetic nervous system that affects young children).

❖ Tumor suppressor genes

Tumour suppressor gene, also called antioncogene, any of a class of genes that are normally involved in regulating cell growth but that may become cancer-causing when damaged. Tumour suppressor genes encode for proteins that are involved in inhibiting the proliferation of cells, which is crucial to normal cell development and differentiation. Because of this ability, tumour suppressor genes can also act to stem the uncontrolled growth of cancer cells. Genetic damage, or mutation, that occurs to these genes contributes to the development of a cancerous tumour.

Cancer research has led to the identification and characterization of many tumour suppressor genes. In 1971 American researcher Alfred Knudson, Jr., postulated that a rare form of eye cancer called retinoblastoma is caused by mutations in a gene designated *RB*. Subsequent research revealed that mutations in this gene also play a role in cancers of the bone, lung, breast, cervix, prostate, and bladder. A number of other tumour suppressor genes (such as *TP53*, which encodes a protein known as p53) have been identified. The mutated form of *TP53* has been implicated in more than 50 percent of all cancers. Mutations in two other tumour suppressor genes, *BRCA1* and *BRCA2*, are associated with an increased susceptiblity to breast cancer; they are found in 5 to 10 percent of all cases and in about 85 percent of all cases of inherited breast cancer.

The tumour suppressor genes in a healthy cell work together with another class of genes, called proto-oncogenes, to control cell reproduction. Tumour suppressor genes code for proteins that restrain cell growth, and proto-oncogenes specify proteins that stimulate cell growth. Mutations in either type of gene can disrupt the delicate balance between inhibition and activation of the molecular processes that regulate a cell's life cycle, leading to the uncontrolled cell growth characteristic of cancer. A mutation in one gene alone does not cause a malignant tumor to develop; a number of genetic insults occurring in a few different genes over time are necessary for a cell

to undergo transformation to a malignant state. For example, a proto-oncogene becomes a cancer-causing oncogene when mutated in a manner that increases the cell's propensity to divide excessively. In order for a cell to give rise to cancer, other mutations, such as damage to a tumour suppressor gene, must arise.

Usually many decades are required for mutations leading to cancer to be accumulated. Cases do occur, however, in younger individuals, many of which are believed to result from a mutation passed from parent to child. Some forms of cancer are associated with an inherited mutation in a tumor suppressor gene. One such familial syndrome, called familial adenomatous polyposis, is a condition in which tumors of the colon arise due to an inherited mutation in the tumor suppressor gene *APC*.