

CANCER AND VIRUSES

CELL CYCLE

The cell cycle is an ordered set of events culminating in cell growth and division into two daughter cells. Non-dividing cells not considered to be in the cell cycle. The stages are G1-S-G2-M. The G1 stage stands for "GAP 1". The S stage stands for "Synthesis". This is the stage when DNA replication occurs. The G2 stage stands for "GAP 2". The M stage stands for "mitosis", and is when nuclear (chromosomes separate) and cytoplasmic (cytokinesis) division occur. Each cell cycle consists of two phases called the interphase and the M phase. The interphase is further separated into three phases of a period of DNA replication or S phase, which separates two growth phases, or G1 and G2 phases. The duration of interphase may be very short and consists only of the time period necessary for DNA replication, such as the case of embryonic cells. On the other hand some cells are forever locked in interphase, such as differentiated cells like skeletal muscle and neurons. These cells are said to be in G0 state, to distinguish from the G1 phase, which implies the cell will eventually undergo DNA replication. The typical adult mammalian cells that undergo cell division generally have cell cycles of 12-36 hrs, with most of the variability during the G1 phase. In contrast when cells divide, the M-phase or the mitotic phase generally lasts 30-60 minutes, regardless of the cell type. Cyclins are cytoplasmic proteins, which stimulate transition of a cell from G1 to S and also entry into M phase. The cyclins are found complexed with specific protein kinases. The complex of a cyclin and a protein kinase is called a maturation promotion factor (MPF) that is a dimer of a protein kinase and cyclin. Because this factor is the same as the one controlling mitosis, MPF is now also called mitosis promoting factor. Cdk (cyclin dependent kinase, adds phosphate to a protein), along with cyclins, are major control switches for the cell cycle, causing the cell to move from G1 to S or G2 to M.

CANCER AND TUMOR SUPPRESSOR GENES

The single most important characteristic of a cancer cell in the body or a culture dish is the loss of growth control. The rate of growth and division is not appreciably different between normal and cancer cells. However, normal cell growth and division are responsive to stimulatory and inhibitory influences in the environment, but cancer cells often behave independent of the influences. There are a large number of structural and biochemical differences between normal and cancer cells, but differences between cancer cells make a "typical" cancer cell impossible to describe. Some of the most striking phenotype of a cancer cell is the transformation within the chromosomes. Normal cells are fastidious in maintaining their normal chromosome complement during growth and division, but cancer cells often have highly aberrant numbers of chromosome or a condition called aneuploidy. The cytoskeleton of normal cells generally contain well organized arrays of microtubules, microfilaments and intermediate filaments; but cancer cells often have reduced and disorganized arrays of cytoskeletal elements. Cancer cells often express different membrane proteins, which changes the adhesivity of the cells. The loss of adhesivity is reflected in the increased motility of cancer cells. Because of the loss of response to inhibition of growth by neighboring cells and the increased motility, cancer cells in culture often overgrow each other rather than remain in a monolayer.

A number of diverse **chemicals, ionization radiation, and DNA and RNA viruses** have been shown to be able to cause cancer and referred to as carcinogens. All of these agents have a common property of causing changes in the genome, but such changes alone are usually insufficient for the development of cancer. The transformation of cells to the cancer state usually occurs with two distinct phases of initiation and promotion. Since gene mutations are stable and inheritable, an initial exposure to a mutagenic substance (initiator) may not be sufficient for transformation, but a subsequent treatment (promoter) that stimulates proliferation may then result in tumor formation.

The genes that have been linked directly with carcinogenesis fall into two classes of tumor suppressor genes and oncogenes. Tumor suppressor genes encode proteins that restrain cell growth and are part of the negative control of cell cycle regulation. There are two well studied examples of tumor suppressor genes: **retinoblastoma (Rb) and p53**. The retinoblastoma tumor develops in young children from neuroblasts of the developing retina. Neuroblasts are embryonic neural cells undergoing rapid rounds of cell division, thus its growth regulation is susceptible to any perturbation. If a neuroblast escapes regulation then it would give rise to a tumor mass. The gene whose loss thus appears to be critical for development of the cancer is called the retinoblastoma or Rb gene. In normal healthy individuals with two good Rb genes, even if one should spontaneously inactivate, no harm is done. Retinoblastoma is a hereditary cancer and in individuals with an inherited mutant Rb gene, the inactivation of the good gene copy would now lead to tumor formation. The gene product has been cloned, sequenced and identified. It is a protein expressed in all cells that bind **transcription factors (E2F)** regulating DNA replication and gene expression. During the cell cycle Rb protein is phosphorylated by Cdk to release its binding of E2F and permit the G1 to S transition and replication occurs, but this regulation is lost in mutant cells that do not express this gene product. Since the Rb gene product is a general cell cycle regulator, one would suspect it to be associated with other cancers and indeed the loss of Rb is now associated with many different cancers.

A second tumor suppressor gene is **p53**. Individuals who inherit only one functional copy of p53 are also predisposed to cancer. The protein product has also been identified and it regulates the expression of another protein (p21), a key kinase that inhibits the cdc2 kinase. This prevents cells from prematurely entering S phase, especially if the DNA has lesion. Thus DNA damage will stimulate the production of p53 to arrest the progression of the cell cycle until the lesion is repaired. If the DNA damage is too severe, the p53 protein directs the cell toward apoptosis or cell death. If the cell lacks functional p53 protein and the cell is able to survive the accumulated gene damage, they progress toward an increasing malignancy. This loss of correlation between complete DNA replication and cell division may explain the aneuploidy of many cancers.

CLASSES OF TUMOR VIRUSES

Viruses and cancer: Much of what we know about the genes involved in the development of cancer is attributable to research into DNA and RNA viruses. There are seven families of viruses associated with tumors (1 RNA and 6 DNA families). The DNA viruses include the hepadnaviruses, the polyomavirus, the papillomaviruses, the adenoviruses, the herpesviruses, and the poxviruses. The RNA virus family is the retroviruses (sub group oncoviruses).

VIRAL TRANSFORMATION: The changes in the biological functions of a cell that result from REGULATION of the cell's metabolism by viral genes and that confer on the infected cell certain properties characteristic of NEOPLASIA. TRANSFORMATION Among the many altered properties of

the TRANSFORMED CELL are: Loss of growth control (loss of contact inhibition in cultured cells). Tumor formation. Mobility. Reduced adhesion. Transformed cells frequently exhibit chromosomal aberrations.

ONCOGENE: A gene that codes for a protein that potentially can transform a normal cell into a malignant cell. An oncogene may be transmitted by a virus in which case it is known as a VIRAL ONCOGENE

DNA TUMOR VIRUSES

In **permissive** cells these viruses produce infectious progeny (lytic life cycle). In cells **non-permissive** for replication the viral DNA can often integrate into the cell chromosomes (usually but not always) at random sites. A typical example is papovaviruses where only the early regulatory proteins such as large-T will be expressed in non-permissive cells from a copy of the viral genome integrated within the cellular genome.

Papovaviridae – Papovaviruses:

1) PAPILOMAVIRUSES: Although there are more than 50 different types of papilloma viruses, not all are associated with cancers. Vulvar, penile and cervical cancers 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**.

2) POLYOMA VIRUSES: Simian virus 40. SV 40 virus causes sarcomas in juvenile hamsters. **Polyoma virus** causes leukemias in mice. After integration into host DNA, only **EARLY FUNCTIONS** are transcribed into mRNA and expressed as a protein product. These are the **TUMOR ANTIGENS**.

3) Adenoviridae-ADENOVIRUSES: These viruses are highly oncogenic in animals. Only a portion of the virus is integrated into host genome. This portion codes for early functions (E1A region contains the oncogenes that code for several tumor antigens). No human cancers have been unequivocally associated with adenoviruses. **E1A** gene product (early non-structural protein) binds to the tumor suppressor protein pRb, while the early protein E1B binds to the tumor suppressor protein p53. Again, when these viruses infect non-permissive for lytic replication cells, they can integrate part of their genome into cellular genomes and express early proteins such as E1A and E1B.

4) Herpesviridae- HERPESVIRUSES

Epstein-Barr virus (infectious mononucleosis; “kissing disease”: This virus is associated with Burkitt's lymphoma, nasopharyngeal cancer, B cell lymphomas in immune suppressed individuals (such as in organ transplantation or HIV) and 5Hodgkin's lymphoma. EBV can cause lymphoma in Marmosets and transform human B lymphocytes in vitro.

Kaposi's sarcoma associated Herpesvirus (KSHV or HHV-8). This virus is intimately associated with Kaposi's lesions. The virus carries a number of genes that can promote tumor formation including chemokine genes and lymphokine analogs.

5) Hepadnaviridae

HEPATITIS B VIRUS: This virus is intimately involved with liver cirrhosis. Liver regeneration after destruction by the virus is thought to promote tumor formation. **The viral X gene**, which is a potent trans-activator of cellular genes is suspected to be involved in cancer formation.

INACTIVATION OF TUMOR SUPPRESSOR PROTEINS:

IN ADDITION TO THEIR ABILITY TO INTEGRATE INTO CELLULAR GENOMES, CODE FOR VARIOUS PROTEINS THAT AFFECT CELLULAR GROWTH, ETC., DNA VIRUSES SPECIFICALLY TARGET AND INACTIVATE TUMOR SUPPRESSOR PROTEINS pRB AND p53.

Interaction of viral proteins coded for by DNA viruses with tumor suppressor genes. Regulatory proteins of **SV 40(large-T)**, **adenovirus(E1a, E1b)** and **papilloma virus (E6, E7)** bind to tumor suppressor genes, cause their proteolytic destruction and therefore, inhibit their normal functions and cause cellular transformation and oncogenesis.

SV40-large T, adenovirus E1A and papilloma virus E7 proteins bind the tumor suppressor protein pRB. This binding releases the **transcriptional factor E2F**, which activates the transcription of many genes and forces cells to go through additional cycles (continue to proliferate).

SV40=large T, adenovirus E1B and papilloma virus E6 proteins bind the p53 tumor suppressor protein and inactivate it. As a result, **p53 can not bind to DNA** and initiate transcription of genes that stop the cell cycle and/or induce apoptosis.

RNA TUMOR VIRUSES (RETROVIRUSES)

- 1) **ONCOVIRINAE:** tumor viruses and those with similar morphology. First discovered was Rous sarcoma virus (RSV)- a slow neoplasm in chickens.

Human tumor viruses: HTLV-1 (human T-cell lymphotropic virus): Adult T-cell leukemia (Sezary T-cell leukemia). HTLV-2: Hairy cell leukemia

- 2) **LENTIVIRINAE:** HIV which causes AIDS belongs to this group. It is much more closely related to some Lentivirinae than it is to HTLV-I and HTLV-II which are oncovirinae
- 3) **Spumavirinae:** There is no evidence of pathological effects of these viruses.

ONCOGENES IN RETROVIRUSES

In retroviruses, these were first discovered as an **extra gene** in **Rous sarcoma virus (RSV)**. This gene was called **src** (for sarcoma). *src is not needed for viral replication*. It is an **extra gene** to those (gag/pol/env) necessary for the continued reproduction of the virus. Many oncogenes have been described by a number of laboratories. Note that they are referred so by a three letter

code (e.g. *src*, *myc*) often reflecting the virus from which they were first isolated. Some viruses can have more than one onc (e.g. *erbA*, *erbB*). Here are a few of the most studied:

Rous sarcoma virus v-src

Simian sarcoma virus v-sis

Avian erythroblastosis virus v-erbA or v-erbB

Kirsten murine sarcoma virus v-kras

Moloney murine sarcoma virus v-mos

MC29 avian myelocytoma virus v-myc

CELLS ALSO HAVE ONCOGENES

The cellular homologs of viral oncogenes are called **proto-oncogenes or c-oncs**, while **viral oncogenes that originated from cellular oncogenes are called v-oncs**. C-oncs are not *identical* to their corresponding v-oncs. After the gene was picked up by the virus it has been subject to mutation, which generally has made it a more potent promoter of cellular growth.

CHARACTERISTICS OF CELLULAR PROTO-ONCOGENES

Protooncogenes are **typical cellular genes involved in cellular growth and cellular regulation**.

Most c-oncs are expressed by the cell at least on some occasions, often when the cell is growing, replicating and differentiating normally. They are usually proteins involved in growth control.

Some transforming retroviruses do not have v-oncs. An example is **avian leukosis virus (ALV)**.

ALV can integrate into the cell genome at many different sites but, **in ALV-induced tumors**, the virus is **ALWAYS** found in a similar position. In all cases of ALV-induced tumors, the viral genome is inserted near a cellular gene called **c-myc**. Thus, inserting the genome of ALV and other chronically transforming retroviruses next to a c-onc has the same effect as carrying in a v-onc (**oncogenesis by promotor insertion**).

Some functions of protooncogenes.

- 1) Control of DNA transcription (found in nucleus): **myc**.
- 2) Signalling of hormone/growth factor binding such as a tyrosine kinase: **src** is a membrane bound tyr **kinase**.
- 3) GTP-binding proteins (GP): **ras**. Again may be involved in signal transduction from a surface receptor to the nucleus
- 4) Growth factors (GF): **sis** is an altered form of platelet-derived growth factor B chain
- 5) Growth factor receptors (REC): **erb-B** is a homolog of the epidermal growth factor receptor (it is also a tyrosine kinase).