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ONCOGENESIS

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ONCOGENESIS

Oncogenesis is the process of malignant transformation leading to the formation of a tumor (tumorigenesis). It is characterized by a progression of changes on cellular and genetic level that ultimately reprogram a cell to undergo uncontrolled cell division, thus forming a malignant mass. Cell division is a physiological process that occurs in almost all tissues and under many circumstances. Under normal circumstances, the balance between proliferation and programmed cell death, usually in the form of apoptosis, is maintained by tightly regulating both processes to ensure the integrity of organs and tissues. Mutations in DNA that lead to cancer (only certain mutations can lead to cancer and the majority of potential mutations will have no bearing) disrupt these orderly processes by disrupting the programming regulating the processes.

Carcinogenesis is caused by this mutation of the genetic material of normal cells, which upsets the normal balance between proliferation and cell death. This results in uncontrolled cell division and the evolution of those of those cells by natural selection in the body. The uncontrolled and often rapid proliferation of cells can lead to benign tumors; some types of these may turn into malignant tumors (cancer). Benign tumors do not spread to other parts of the body or invade other tissues, and they are rarely a threat to life unless they compress vital structures or are physiologically active, for instance, producing a hormone. Malignant tumors can invade other organs, spread to distant locations (metastases) and become life-threatening.

More than one mutation is necessary for carcinogenesis. In fact, a series of several mutations to certain classes of genes is required before a normal cell will transform into a cancer cell. Only mutations in certain types of genes which play vital roles in cell division, apoptosis (cell death), and DNA repair will cause a cell to lose control of its cell proliferation.

THE NORMAL CELL CYCLE

The resting (non-dividing) cells in the body are recruited into the cell cycle and pass through different phases that eventually lead to cell replication. The phases are G₀, G₁, M, G₂ and S phases. The activities that take place in each phase are G₀ (cells not actively proliferating, thus resting with no activity), G₁ (cells already recruited and are in a gap phase before DNA synthesis), M (mitosis), G₂ (gap phase between DNA synthesis and mitosis) and S (DNA synthesis).

The orderly progression of cells through the various phases of cell cycle is under the control of cyclins and cyclin-dependent kinases (CDKs) and their inhibitors. CDKs drive the cell cycle by phosphorylating critical target proteins that are required for progression of the cells to the next phase of the cycle. CDKs are expressed during the cell cycle though in an inactive form. They become activated by phosphorylation after binding to the family of proteins

called cyclins. Cyclins in contrast to CDKs are synthesized during specific phases of the cell cycle with the function to activate the CDKs. On completion of this task, cyclin levels decline rapidly. Over 15 cyclins have been identified; cyclin D, E, A and B appear sequentially during the cell cycle and bind to one or more CDKs.

CYCLIN D AND RB PHOSPHORYLATION

Cyclin D is the first cyclin to increase in the cell cycle; it appears in the mid G1 phase but has become undetectable in S phase. It exists in three forms i.e. D1, D2 and D3 but are generally referred to as Cyclin D. LIKE OTHER CYCLINS, Cyclin D is unstable and is degraded via the ubiquitin-protease pathway. During the G1 phase, it binds to and activates CDK4 forming a cyclin D-CDK4 complex. This complex is responsible for phosphorylation of the retinoblastoma susceptibility protein (RB). This process is a molecular ON-OFF switch for the cell cycle. In the phosphorylated state, RB prevents cells from replicating by forming a tight inactive complex with the transcription factor E2F (which is a family of transcription factor). RB phosphorylation dissociates the complex and releases the inhibition on E2F transcriptional activity. In other words, phosphorylation of RB eliminates the main barrier to cell-cycle progression and promotes cell replication.

The molecular basis of the braking effect of RB on the cell cycle is such that hypophosphorylated RB which is present in quiescent cells in G0 and early G1 phase binds to a protein complex that contains E2F and a subunit called DP1. The E2F/DP1/RB binds to promoters of E2F-responsive genes such that the genes are silent because RB recruits histone deacetylase which is an enzyme that causes compaction chromatin and inhibition of transcription. On stimulation by growth factors, the concentration of cyclin D and E in quiescent cells go up resulting in activation of cyclin D-CDK4 and cyclin E-CDK2 at the G1/S restriction point thus causing phosphorylation of RB. Hyperphosphorylated RB dissociates from the complex, activating the transcription of E2F target genes that are essential for the progression through the S phase. These include cyclin E, DNA polymerases, thymidine kinase, dihydrofolate reductase and several others. During the M phase, the phosphate groups are removed from RB cellular phosphatases, thus regenerating the hypophosphorylated form of RB.

CELL-CYCLE PROGRESSION BEYOND THE G1/S RESTRICTION POINT

Further progression through the S phase and the initiation of DNA replication involves the formation of an active complex between cyclin E and CDK2. Activated E2F increases the transcription of cyclin E and of polymerases needed for DNA replication, thus stimulating DNA synthesis.

The next important point is the G2/M transition. This is initiated by the E2F-mediated transcription of cyclin A, which forms the cyclin A-CDK2 complex that regulates events at the mitotic prophase. The main mediator that propels the cell beyond prophase is the cyclinB-CDK1 complex which is activated by a protein divided cells

phosphatase (Cdc 25) and begins to accumulate in the nucleus from early prophase. Cyclin B-CDK1 causes the breakdown of the nuclear envelope and initiates mitosis. Complexes of CDKs with cyclins A (cyclin A exist in two isoforms A1 and A2; A2 is essential in the cell cycle) and B regulate some critical events at the G2/M transition, such as the decrease in microtubule stability, the separation of centrosomes and chromosome condensation. Exit from mitosis requires the inactivation of cyclin B-CDK1. Newly divided cells can then return to G1 and initiate a new replicative cycle or go into quiescence. The absence of both isoforms of cyclin E (E1 and E2) prevents quiescent cells from entering the cell cycle.

CELL-CYCLE INHIBITORS

The activity of cyclin-CDK complexes is tightly regulated by inhibitors called CDK inhibitors. There are two main classes of CDK4 inhibitors: Cip/Kip and the INK4/ARF families. These inhibitors function as tumor suppressors and are frequently altered in tumours. The Cip/Kip family has three components namely p21, p27 and p53; which bind to and inactivate the complexes formed between cyclins and CDKs. Transcriptional activation of p21 is under the control of p53, a tumour suppressor gene that is that of surveillance, triggering check point controls that slows down or stop cell-cycle progression of damaged cells or causes apoptosis. The human INK4a/ARF locus (a notation for "inhibitor of kinase 4/alternative reading frame") encodes two proteins p16INK4a and p14arf which block the cell cycle and act as tumour suppressors. P16INK4a competes with cyclin D for binding to CDK4 and inhibits the ability of the cyclin D-CDK4 complex to phosphorylate RB thus causing cell cycle arrest at late G1. It is frequently mutated or inactivated by hypermethylation. The INK4a locus encodes a second gene product p14ARF (p19ARF in mice) which act on p53. Though both p16INK4a and p14ARF block the cell-cycle, their targets are different; p16INK4a acts on cyclin D-CDK4 while p14ARF prevents p53 degradation.

CELL-CELL CHECKPOINTS

The cell-cycle has its own internal control called checkpoints. There are two main checkpoints: these are at the G1/S and G2/M points. The phase is the point of no return in the cell cycle; thus before a cell makes its final commitment to replicate, the G1/S checkpoint must check for DNA damage. If DNA damage is detected, the DNA repair machinery and the mechanism that arrest the cell-cycle are set in motion to kill the cell. The G2/M checkpoint monitors the completion of DNA replication and checks if the cell can safely initiate mitosis and separate chromatids. This is very important in cases of cell damage by radiation; the checkpoint is activated and the cycle is arrested in G2. To function effectively, cell-cycle checkpoints require sensors of DNA damage, signal transducers and effector molecules. Arrest in G2/M point involves both p53 dependent and p53 independent mechanisms. Defect in cell-cycle checkpoint components is a major cause of genetic instability in cancer cells.

CAUSES OF CANCERS

Causes of cancer are varied and numerous; and population-based studies has identified certain agents that are either causative or predisposed to its formation,

CARCINOGENS

Carcinogens are chemical, physical or biological agents whose continued exposure causes cellular damage which leads to cancer formation. These include:

TOBACCO SMOKING

Smoking is associated with cancers of the lungs, oesophagus, respiratory tract, bladder, pancreas and possibly stomach, kidneys and liver. The risk increases with the number of cigarette sticks smoked per day, the tar content of the cigarette and duration of smoking in years. Early starters have an increased risk. Individuals who do not smoke directly but are close to the smokes of others who smoke cigarette are equally vulnerable. These are called passive smokers. Nonsmoking spouses of smokers are 30% more likely to develop cancer than those with nonsmoking partners. Children of smokers and those working in smoky environment are at higher risk. In addition, cigars, pipes and smokeless tobacco have been implicated in increased risk of cancer. Oral cancers are commoner in people who use smokeless tobacco or snuff. Snuff users are 50 times more likely to develop cancers of the cheek or gum than nonusers.

DIET

Dietary indiscretion has been implicated in cancers. Saturated fats and high fatty dairy products have been implicated. Adult obesity increases risk of endometrial cancer in women and appears to increase risk of breast, colon, kidney and gallbladder cancers. Alcohol consumption especially with smoking is important in oesophageal and gastric cancers.

ONCOGENIC PATHOGENS

These include viruses, bacteria and parasites. Viruses like human papilloma virus (in cancer of cervix), hepatitis B and C (in liver cancer), Epstein-Barr virus (in cancer of lymphatic system) and human immunodeficiency virus (HIV) in opportunistic cancers. Bacteria like helicobacter pylori have been implicated in gastric cancer. In developing countries, parasites like the liver fluke clonorchis sinensis (in liver cancer) and Schistosoma haematobium (in bladder cancer) are important.

RADIATION

Exposure to x-rays, ultraviolet rays, electromagnetic waves and excessive exposure to sunlight are important in sunburns and subsequently cancers of the skin. Occasionally, therapeutic use of radiation in treatment can cause secondary cancers.

ENVIRONMENTAL AND INDUSTRIAL CHEMICALS

Pollution of air, water and soil by industrial chemicals have been associated with occurrence of certain cancer types in certain localities.

HEREDITARY FACTORS

Inherited genetic mutations have been identified in certain cancers. These are called familial cancers; and include breast cancer (BRCA1 and BRCA2), ovarian cancers as well as the Lynch II Syndrome, there is colorectal cancer in association with other forms of cancers like endometrial, ovarian, gastric and urinary tract tumours; it is associated with a greater risk of recurrence.

STEROID HORMONES

Studies suggest that reproductive tract cancers may be affected by naturally occurring hormones in the body. Relatively high or prolonged exposure to female sex hormones like estrogen has been implicated in breast and endometrial cancers. These may manifest as early menarch and late menopause, never having children and hormone replacement therapy.

DEMOGRAPHIC CHARACTERISTICS

Studies have shown that age, race, ethnicity and gender affect an individual's chance of developing cancers. Most cancers occurs in middle aged adults or older. It is due to accumulation of genetic mutations over a period of years which eventually manifest as cancers. Men are likely to develop cancer than women but the reason for the difference is not yet established.

MECHANISMS OF CARCINOGENESIS

Cancer is a genetic disease: in order for cells to start dividing uncontrollably, genes that regulate cell growth must be damaged. Proto-oncogenes are genes that promote cell growth and mitosis, whereas tumor suppressor genes discourage cell growth, or temporarily that cell division to carry out DNA repair. Typically, a series of several mutations to these genes is required before a normal cell transforms into a cancer cell (Fig. 3.1).

PROTO-ONCOGENES

Proto-oncogenes promote cell growth in variety of ways. Many can produce hormones, "chemical messengers" between cells that encourage mitosis, the effect of which depends on the signal transduction of the receiving tissue or cells. Some are responsible for the signal transduction system and signal receptors in cells and tissues themselves, thus controlling the sensitivity to such hormones. They often produce mitogens, or are involved in transcription of DNA in protein synthesis, which create the proteins and enzymes which are responsible for producing the products and biochemicals cells use and interact with.

Mutations in proto-oncogenes can modify their expression and function, increasing the amount or activity of the product protein. When this happens, they become oncogenes, and, thus, cells have a higher chance to divide excessively and uncontrollably. The chance of cancer cannot be reduced by removing proto-oncogenes from the genome, as they are critical for growth, repair and homeostasis of the body. It is only when they become mutated that the signals for growth become excessive. It is important to note that a gene possessing a growth-promoting role may increase carcinogenic potential of a cell, under the condition that all necessary cellular mechanisms that permit growth are activated. This condition includes also the inactivation of specific tumor suppressor genes. If the condition is not fulfilled, the cell may cease to grow and can proceed to die. This makes knowledge of the stage and type of cancer cell that grows under the control of given oncogene crucial for the development of treatment strategies.

TUMOR SUPPRESSOR GENES

Tumor suppressor genes code for anti-proliferation signals and proteins that suppress mitosis and cell growth. In general, tumor suppressors are transcription factors that are activated by cellular stress or DNA damage. Often DNA damage will cause the presence of free-floating genetic material as well as other signs, and will trigger enzymes and pathways that lead to the activation of tumor suppressor genes. The functions of such gene is to arrest the progression of cell cycle in order to carry out DNA repair, preventing mutations from passing on to daughter cells. Canonical tumor suppressors include the p53 gene, which is a transcription factor activated by many cellular stresses including hypoxia and ultraviolet radiation damage.

However, a mutation can damage the tumor suppressor gene itself, or the signal pathway that activates it, switching it off. This can hinder DNA repair, so that DNA damage accumulates, including changes that lead to cancer

MULTIPLE MUTATIONS

In general, mutations in both types of genes are required for cancer to occur. For example, a mutation limited to one oncogene would be suppressed by normal mitosis control and tumor suppressor genes, first hypothesized by the Knudson hypothesis. A mutation to only one tumor suppressor gene would not cause cancer either, due to the presence of many "backup" genes that duplicate its functions. It is only when enough proto-oncogenes have mutated into oncogenes, and enough in tumor suppressor genes deactivated or damaged, that the signals for cell growth overwhelm the signals to regulate it, that the cell growth quickly spirals out of control. Often, because these genes regulate the processes that prevent most damage to genes themselves, the rate of mutations increases as one gets older, because DNA damage forms a feedback loop. Usually, oncogenes

are dominant alleles, as they contain gain-of-function mutations, whereas mutated tumor suppressors are recessive alleles, as they contain loss-of-function mutations. Each cell has two copies of the same gene, one from each parent, and, under most cases, gain of function mutation in one copy of a particular proto-oncogene is enough to make that gene a true oncogene, while usually loss of function mutation must happen in both copies of a tumor suppressor gene to render that gene completely non-functional. However, cases exist in which one loss of function copy of a tumor suppressor gene can render the other copy non-functional, called the dominant negative effect. This is observed in many p53 mutations.

Mutation of tumor suppressor genes that are passed on to the next generation of not merely cells, but their offspring, can cause increased likelihoods for cancers to be inherited. Members within these families have increased incidence and decreased latency of multiple tumors. The mode of inheritance of mutant tumor suppressors is that the affected member inherits a defective copy from one parent, and a normal copy from another. Because mutations in tumor suppressors act in a recessive manner (note, however, there are exceptions), the loss of the normal copy creates the cancer phenotype. For instance, individuals that are heterozygous for p53 mutations can develop retinoblastoma. In similar fashion, mutations in the adenomatous polyposis coli gene are linked to adenomatous polyposis coli, with thousands of polyps in the colon while young, whereas mutations in BRCA1 and BRCA2 lead to early onset of breast cancer.

NON-MUTAGENIC CARCINOGENS

Many mutagens are also carcinogens, but some carcinogens are not mutagens. Examples of carcinogens that are not mutagens include alcohol and estrogen. These are thought to promote cancers through their stimulating effect on the rate of cell mitosis. Faster rates of mitosis increasingly leave less opportunities for repair enzymes to repair damaged DNA during DNA replication, increasing the likelihood of a genetic mistake. A mistake made during mitosis can lead to the daughter cells receiving the wrong number of chromosomes, which leads to aneuploidy and may lead to cancer.

ROLE OF VIRAL INFECTIONS

Furthermore, many cancers originate from a viral infection; this is especially true in animals such as birds, but less so in humans. 12% of human cancers can be attributed to a viral infection. The mode of virally-induced tumors can be divided into two, acutely-transforming or solely-transforming. In acutely-transforming viruses, the viral particles carry a gene that encodes for an overactive oncogene called viral-oncogene (v-onc), and the infected cell is transformed as soon as v-onc is expressed. In contrast, in slowly-transforming viruses, the virus genome is inserted, especially as viral genome insertion is obligatory part of retroviruses.

near a proto-oncogene in the host genome. The viral promoter or other transcription regulation elements, in turn, induces uncontrolled cellular proliferation. The viral genome insertion is not specific to proto-oncogenes and the chance of insertion near that proto-oncogene is low. Slowly-transforming virus, already carries the viral-oncogene.

Table 2

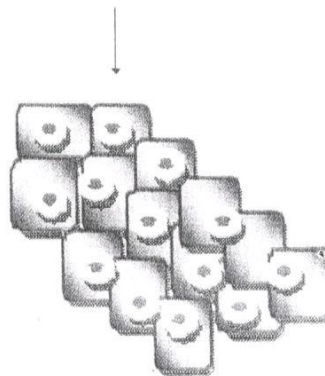
Oncogene

Mechanism

Mutation inactivates tumor suppressor gene



CELLS PROLIFERATE



Mutation inactivates DNA repair gene

Mutation of proto-oncogene creates an oncogene

Mutation inactivates several more tumor suppressor gene

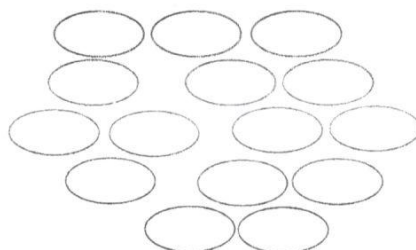


Fig 3.1 Mechanisms of carcinogenesis

A SIMPLIFIED SCHEME OF CANCER PATHOGENESIS

Cancers are caused by a series of mutations. Each mutation alters the behavior of the cell somewhat. (Table 3.1)

Table 3.1 Scheme of Cancer Pathogens

Oncogenesis		
Mechanism	Action	Example
Growth	Overexpression of growth factor	c-erb-B2
Promotion	Receptors (such as epidermal growth factor, or EGF) making cells more sensitive to growth stimuli	Ras
	Overexpression of a gene product by stimulation from an oncogene (such as ras)	c-sis
	Lack of normal gene regulation through translocation of a gene where it is controlled by surrounding genes to a place where it is no longer inhibited	c-abl
	Binding of oncogene product to the nucleus with DNA transcriptional activation to promote entry into the cell cycle	c-mycl
Loss of tumor suppressor	Loss of normal growth inhibition	BRCA-