DNA Mutation

Cancer occurs due to genetic abnormalities either acquired (somatic mutation) or inherited from a parent.

The two characteristics of cancer cells are their heritable ability to proliferate despite the normal constraints which inhibit cell proliferation, and their ability to invade neighbouring tissues.

For these properties to be passed on to cell progeny, they must be genetically encoded, and cancer cells demonstrate changes in their genetic material compared to the normal cell.

Damage to DNA can be acquired due to exposure to carcinogens, or result from mistakes in DNA replication. In addition inherited defects can be passed on from parental sources (germ-line mutations).

Acquired genetic damage (**somatic mutations**) can be caused by exposure to environmental carcinogens, or in some cases viral infection.

Environmental carcinogens include UV light, cigarette smoke, asbestos and food additives.

Single DNA mutations do not usually give rise to cancer. Mutations in more than one gene are required for a cell to develop cancerous properties.

In somatic cells that frequently divide there is less time for DNA repair to occur, prior to DNA replication.

These cells such as bone marrow hematopoetic cells, skin cells, colonic epithelia, are therefore more susceptible to the accumulation of genetic mutations, and the development of cancer.

In addition with increasing age DNA repair mechanisms wane and the cumulative exposure to carcinogens and environmental exposure to various factors results in DNA damage which may not be adequately repaired.

DNA replications errors

In the course of a human lifetime, an estimated 1016 cell divisions will take place. During each cell division the genetic material of the cell is replicated in a process that is not without error.

The rate of mutation caused by the limited accuracy of DNA replication and repair alone is estimated at 10-6 mutations per gene per cell division.

It follows that each gene is likely to be affected on more than one occasion, with mutations resulting in varying degrees of disruption to the gene product. The erroneous insertion of an incorrect nucleotide during DNA replication may have no effect on the gene product, however, where the codon changes to a different amino acid, or a stop codon, the function of the gene product can be seriously affected.

When a disruptive mutation occurs in a gene involved in the regulation of cell division, the cell may acquire the ability to proliferate independently of normal cell cycle controls. As mutations caused by errors in DNA replication and repair accumulate in the somatic tissue, and the ability of the cell to accurately repair DNA damage decreases during aging, the chance of cancer developing increases with age.

Irradiation

Radiation, including UV light and ionizing radiation from X-rays and radioactive decay, also causes cumulative DNA damage.

Ionizing radiation induces DNA damage directly or indirectly through the production of free radicals.

Single and double-strand breaks to the DNA double helix occur as a result of exposure to ionizing radiation.

Irradiation (cont)

As compared to other types of DNA damage, double-strand breaks are intrinsically more difficult to accurately repair through homologous recombination, or non-homologus end joining as no template exists for correct rejoining.

The repair processes can cause induction of gene mutations and subsequently promote cancer development.

UV radiation is absorbed by the DNA molecule, which can result in the formation of a thymine dimer. As one of the four bases of the DNA molecule, the dimerisation of adjacent thymine nitrogenous bases disrupts the activity of DNA polymerase during DNA replication and without repair causes induction of mutations.

Chemical carcinogens

Chemical carcinogens (cancer promoting agents) include benzo[a]-pyrene in cigarettes and aflatoxin, a well-characterized toxin produced by a mould that grows on stored grain and peanuts.

The most noxious chemical carcinogen is that found in cigarettes, which is responsible for up to 30% of all human tumours.

Many of the known chemical carcinogens cause direct DNA damage, acting to chemically modify the DNA molecule.

The more potent chemical carcinogens, including the fungal toxin aflatoxin B1, are not specifically reactive with DNA until being activated by metabolic processes involving the cytochrome P-450 oxidases.

These oxidases normally function to inactivate ingested toxins, however, in some cases cause conversion of toxins to highly mutagenic compounds (i.e. compounds which cause mutations in DNA).

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Virus Infection

Viruses have been shown to be the causative agents in a small but significant proportion of human cancers.

Only a limited number of human cancer-inducing viruses have been identified, with these being mainly DNA viruses, with a smaller number of RNA retroviruses also described.

There is often a delay of many years between the initial viral infection and cancer development (this is called latency), so there are likely to be a number of viruses whose tumour association has not yet been identified.

DNA viruses can carry genes that cause subversion of the normal cell cycle leading to uncontrolled host cell proliferation. Retroviruses often cause genetic disruption in genes that promote cancer development following insertion of the viral gene either upstream of the proto-oncogene, or within the coding sequence, leading to cell transformation.

Human T-cell leukemia virus, an RNA virus, causes a rare form of leukemia with high incidence in Japan and the West Indies. Epstein-Barr virus, of the Herpesvirus family, and Papillomavirus are examples of cancer-causing DNA viruses which induce Burkitt's lymphoma and carcinoma of the cervix respectively.

Inherited Cancer Predisposition Syndromes

In some individuals, all of the cells in the body contain an inborn genetic defect, which increases the probability that cancer may develop during the lifetime of the individual.

This type of cancer susceptibility gene is present in the inherited genetic material (germ-line) of the individual that can be passed down from parent to child. Such cancer-prone families often present with similar types of cancers in many members of the family over successive generations.

Inherited Cancer Predisposition Syndromes (cont)

In addition, many of these cancers present at a younger age than that observed for sporadic (random, with no pattern) cancers that occur in the general population.

It has been predicted that 5-10% of all cancers are part of defined inherited cancer syndromes.

Classical inherited cancer syndromes include some cases of breast cancer and colon cancer. In some instances the genes implicated in inherited cancer syndromes are also implicated in the pathogenesis of sporadic cancers.

Familial susceptibility for cancer can occur as inherited cancer susceptibility for a single type of cancer, or for a number of different types of cancer, as part of a familial cancer predisposing syndrome.

Features that suggest an inherited cancer susceptibility gene defect include:

Several close (first degree relatives) with the same cancer; Several close relatives with related cancers - breast and ovarian and endometrial; Two family members with the same rare cancer; Early age of onset of cancer; Bilateral tumours in paired organs; Synchronous or successive tumours; Tumours in two organs in one individual.

Cancer critical genes

Three distinct classes of genes-oncogenes, tumour suppressor genes and DNA mismatch repair genes, when mutated, cause **cell transformation** (change of a normal cell to a cancer cell).

Individuals born with a mutation in any one of these gene types has an inherited predisposition to cancer.

Cancer critical genes (cont)

This most commonly occurs with tumour suppressor genes. However, additional mutations may be required for the onset of cancer development. In inherited cancer predisposition syndromes, genetic defects in these gene/s are present in all cells of the body.

In acquired sporadic cancers the mutation is found only in the cancer cell and its progeny. In sporadic cancers surrounding normal cells and germ line cells show no mutations.

Oncogenes

These genes are expressed in normal cells as "proto-oncogenes" whose normal function is to promote cell growth and division.

In general these gene products act as accelerators of specific phases of the cell cycle during the "G1" or growth phase of the cell.

Over 100 proto-oncogenes have been identified. Much of the original identification of oncogenes has come from studies of tumour viruses.

Proto-oncogenes may be growth factors or their receptors, various intracellular signalling proteins or enzymes, regulators of specific phases of the cell cycle, or transcription factors.

Unlike tumour suppressor genes, which are responsible for a number of familial inherited predisposition syndromes, only one oncogene, ret, has been identified as the cause of a familial cancer syndrome.

Cancers that result from mutations in proto-oncogenes are presumed to arise from acquired DNA damage, as opposed to inherited mutations. Mutations that transform a "proto-oncogene" to an oncogene are normally **"gain of function,"** that is these mutations result in an increase in the levels, or activity of the growth promoting gene product. Only a single allele is required to be mutated for enhanced growth potential.

Cancer critical genes (cont)

There are three principal mechanisms by which a normal cellular proto-oncogene may be converted into an oncogene.

These mechanisms are all “gain of function”, in other words the growth promoting characteristics of these gene products are enhanced. Loss of function mutations of proto-oncogenes will not promote cancer.

1. An activating mutation in a DNA coding sequence**

In this model, minor mutations in the gene result in the expression of the protein at normal levels, but mutations at critical sites result in the protein having increased activity, or for a receptor activity in the absence of ligand-binding, or stimulus.

For example, activating mutations in the Ras gene have been detected in 30% of all human cancers. Oncogenic Ras mutations arise from a point mutation in the coding sequence which result in increased activity. Another example is the Ret proto-oncogene, which is a cell surface tyrosine kinase receptor. Activating mutations at specific sites in the receptor sequence are associated with the clinical syndrome of multiple endocrine neoplasia type 2.

2. **Gene amplification**

In this situation, multiple copies of the gene are expressed, resulting increased production of a signalling protein such as the epidermal growth factor receptor in breast cancer. The increase in copy number can reach up to several hundred fold. Amplification of specific genes such as the transcription factors encoded by the MYC gene family are observed in specific types of tumours.

For example N-myc is amplified in approximately 30% of neuroblastomas and in some cases of lung cancer.

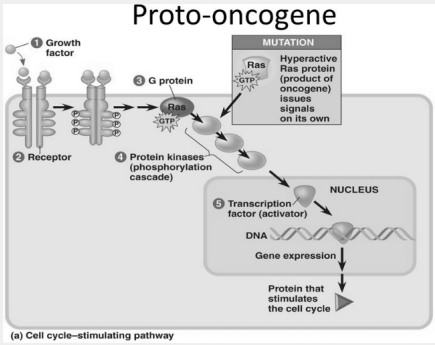
3. **Chromosomal rearrangement.**

Cancer critical genes (cont)

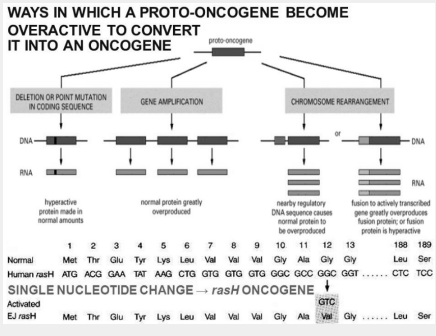
The chromosomal arrangement occurs only in the somatic and not the germ-line tissues and results in the translocation of genetic material from one chromosome to another. Many cases of chromosomal rearrangement leading to human disease are seen in human leukemias and lymphomas. Up to 75% of human leukemias have detectable chromosomal rearrangements in the leukemic, but not the germ line cells.

For example, Bcl-2 is a member of a family of proteins that regulate apoptosis. Bcl-2 was initially discovered by the presence of its translocation in a specific type of human lymphoma. Translocation of the immunoglobulin gene on chromosome 14 with the Bcl-2 gene on chromosome 18, results in persistent overexpression of Bcl-2 and inhibition of cell death

Proto-oncogene products stimulate cell division.



Three ways proto-oncogene converts to oncogene



Tumour suppressor genes

Tumour suppressor genes are defined as genes that sustain loss of function in the development or progression of cancer

Inactivating mutations in tumour suppressor genes may be inherited in the germ line, or acquired by somatic mutation.

In contrast to oncogenic mutations, tumour suppressor gene mutations must affect both copies (alleles) of the gene and must result in loss of function of the gene for cancer to result.

Although many of the originally described tumour suppressor genes were direct regulators of the cell cycle such as Rb and p53, more recently described genes serve other functions. Approximately 20 putative tumour suppressor genes have now been identified, including those that encode cell-cycle regulators, transcription factors, phosphatases and a protein that regulates RNA polymerase II elongation.

Tumour suppressor genes often function at critical points in the control of the cell cycle, cell proliferation, differentiation, and apoptosis and in response to genetic damage.

These genes are normally expressed in all cells and function as “brakes” on the cell cycle and typically act before the synthetic or “S” or synthetic phase of the cell cycle. The S phase checkpoint allows time for DNA repair to occur prior to DNA replication.

In general defects in tumour suppressor genes that result in malignant transformation result in loss of function of the gene, with both copies of the gene affected.

This has led to the **“two hit hypothesis”** of cancer development and has been clearly demonstrated in familial and sporadic cases of Retinoblastoma, mediated by mutations in the tumour suppressor gene Rb.

Tumour suppressor genes (cont)

Retinoblastoma, a rare childhood cancer affecting the retina of both eyes, occurs in certain families at high frequency and at a young age.

Examination of the chromosomes of a cell from an affected child led to the discovery that a small piece from a portion of chromosome 13 was missing. This deletion is present in one allele of all the child's cells and as such likely to result from an inherited deletion.

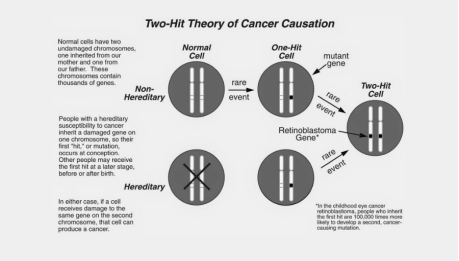
These children inherit a strong predisposition for development of retinal cancer with ninety percent of carriers developing the disease.

Both copies of the Rb gene are required to be affected for cancer to develop. Therefore a second mutation in the remaining normal allele must occur after birth leading to development of cancer.

In addition retinoblastoma also occurs sporadically, i.e. 1 in 30,000, at an older age among members of the larger population as a sporadic cancer. These individuals are born with two normal copies of the Rb gene, and sequentially mutate both Rb alleles leading to cancer.

The RB gene product pRb plays a significant role in the regulation of the cell cycle. During most of the G1 phase of the cell cycle, the E2F transcription factor is bound by pRb, which prevents E2F transcription factor activity inhibiting expression of S phase proteins. Phosphorylation of pRb by cyclin-dependent kinases releases E2F and allows transition of the cell to S phase. When pRb is not present in the cell, due to deletion or mutation, there is no restriction of the cells irreversible entry into S phase and subsequent DNA replication.

"Two-Hit" hypothesis for cancer.



DNA mismatch repair genes

Any induced mutation that affects the ability of the organism to replicate its genetic material without mistake is likely to also have a carcinogenic effect.

Mismatch repair genes encode proteins that cooperate in the removal of erroneously incorporated bases from the nucleotide sequence following DNA replication.

These proteins function to recognise imperfect sequences, remove the surrounding segment of DNA and replacing the sequence with the correct nucleotides.

Mutations in DNA mismatch repair genes that result in an altered gene product cause an increase in the mutation rate, and thus increases the risk of mutation in proto-oncogenes and on tumour suppressor genes.

For example, the HNPPC (human non polyposis coli) gene is a mismatch repair gene which is mutated in some types of inherited colon cancer predisposition syndromes.

Multi-step carcinogenesis

Cancer results from the accumulation of multiple genetic errors affecting both oncogenes and tumour suppressor genes.

The concept of multi-step carcinogenesis is well supported.

This is well shown in colon cancer where ready accessibility to tissue has demonstrated the genetic changes that occur as normal colon epithelium changes from benign polyps to invasive malignant cancer.

Multi-step carcinogenesis (cont)

In colon cancer it appears that seven or more genetic events (mutations in APC, ras, DCC, mismatch repair, TGF-b-R, p53 and others) may be required before an invasive carcinoma develops. By the time a patient develops clinical evidence of cancer, the cancer usually has many genetic mutations in biopsy samples. With increasing time more mutations accumulate as the cancer becomes more malignant and spreads.

p53 Mutation and effects on cancer treatment

One of the reasons why cancer cells survive with a greater advantage than normal surrounding tissue is because these cells have developed molecular mechanisms to evade programmed cell death (apoptosis)

One of the molecular mechanisms mediating the evasion of apoptosis is the acquisition by the cancer cell of a mutation in the tumour suppressor gene p53, which leads to abnormal and inefficient apoptotic responses.

It is noteworthy that more than 50% of all human tumours show mutations and loss of function in the p53 protein.

Apoptosis resistance in cancer cells

It is probable that many cells that are gradually developing a malignant potential are rapidly terminated by apoptotic pathways.

However, in highly malignant tumours, the cell death pathways are modified, or significantly inhibited.

The acquisition of mutations in the p53 gene allows the tumour to evade apoptotic cell death pathways that are present in normal cells. If we could repair the cell death pathways, which are lacking in the cancer cells, theoretically we could induce cancer cells to die and thereby spare the normal surrounding tissues.

p53 Mutation and effects on cancer treatment (cont)

Therefore understanding the mechanisms by which a normal cell lives or dies in response to p53, may help us to develop new strategies to promote regulated cancer cell death.

p53 structure and function

p53 was so named because the molecular mass of the protein is 53 kilodaltons.

p53 is a transcription factor, which contains three specific domains, an N-terminal transactivation domain, a central specific DNA binding core and a C-terminal domain that contains nuclear localization sequences and an oligomerization sequence.

p53 shuttles in and out of the nucleus, regulated by N-terminal and C-terminal regions.

The most common site for p53 mutations in many human cancers is with in the central DNA-binding core.

Two other p53 family members have been identified p63 and p73, these latter 2 proteins regulate normal development, but are not commonly associated with mutations in cancer. p53 is not required for normal development, as shown by p53 knockout mice, which develop normally, but start to develop cancer by 3 months of age.

In normal cells the levels of p53 are extremely low, however, p53 protein levels rapidly increase within the cell following specific stimuli, which include exposure to ultraviolet light, gamma radiation, transformation of cells with oncogenes such as Ras and Myc, and oxygen deprivation.

Elevated levels of p53 may drive damaged cells to commit suicide by apoptosis, if DNA damage is severe.

Alternatively p53 may halt the cell cycle until the DNA damage is repaired.

p53 Mutation and effects on cancer treatment (cont)

The choice of cell response to p53 depends on specific factors such as the cell type, the cell environment, and other oncogenic alterations sustained by the cell.

In general, the effect of p53 activation is to inhibit cell growth, either through cell cycle arrest, or induction of apoptosis.

Collectively these responses prevent tumour development and progression. The rapid cellular increase in p53 levels in response to DNA damage stabilises the p53 protein by specific molecular mechanisms, which lead to an increase in the intracellular p53 levels.

Cellular functions of p53

DNA damage can be induced following exposure to toxins, UV light radiation, and chemicals. The cellular machinery maintains the ability to detect DNA damage and arrest the cell at specific checkpoints, which are known as DNA damage checkpoints.

These classically occur at the G1 checkpoint, which prevents entry of the cell into S-phase and the late G2 checkpoint, which prevents entry of the cell into mitosis.

These DNA damage checkpoints are not essential for normal cell division.

Low-level DNA damage occurs during normal cellular life, and if not corrected DNA errors accumulate. In cells that lack these checkpoints, accumulation of DNA mutations occurs, which may lead to mutation of proto-oncogenes, or tumour suppressor genes, and results in enhanced cellular proliferation.

The G1 checkpoint blocks entry into S phase, which is the phase during which DNA replication occurs. The G1 checkpoint is regulated by a series of specific kinases and phosphatases, which are in turn regulated by p53-mediated transcription of genes including a protein called p21, which regulates cyclin-dependent kinases at this checkpoint.

Cellular functions of p53 (cont)

Thereby p53 regulates the entry of cell into the S-phase.

In addition, p53 levels are regulated by a complex interaction of p53 with the ubiquitin ligase MDM2. In the normal cell MDM2 forms a complex with p53 and targets this protein for degradation in proteosomes. Upon DNA damage p53 is phosphorylated by protein kinases. Phosphorylated p53 has reduced binding to MDM2, leading to decreased degradation of p53 and increased cellular levels of p53. As a consequence, p53 is able to mediate the transcription of a whole host of target genes.

p53 target genes

p53 is a transcription factor that directly activates and regulates the expression of the genes that contain p53 binding sites in their regulatory regions.

In the human genome at least 4,000 potential p53 regulator gene targets have been identified, although it is not clear yet if they will all be genuine physiological p53 targets. Many of these genes that are induced by p53 can be divided into specific classes of protein that either inhibit cell growth, regulate DNA repair, regulate apoptosis, or control angiogenesis, i.e., the formation of new blood vessels.

Proteins regulated by p53 that control apoptosis include genes that are cell-death effectors, i.e., induce cell death, including both death receptor and mitochondrial apoptotic pathways.

p53 can also regulate the expression of genes that inhibit cell survival, such as PTEN, a commonly mutated tumour suppressor gene in breast and brain cancers.

Loss of p53 in human cancers

Mutation of p53 in cancer cells results in loss of apoptotic function, and cell cycle regulation.

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Cellular functions of p53 (cont)

These mutations can occur by a variety of different mechanisms and commonly occur in the DNA binding domain in greater than 95% of cases.

Often the mutations comprise a single point mutation. Mutant p53 proteins are often more stable than normal p53 and may be expressed at extremely high levels in the tumour cell. However, mutant p53 cannot function to mediate apoptosis, or cell cycle arrest.

The mutant p53 may act as a dominant-negative protein, i.e., a non-functional protein, which competes with the normal non-mutated protein, thereby blocking normal p53 activity.

n addition, some of the mutant p53 may acquire new transforming (cancer-promoting) functions, i.e., a gain-function mutant. As a consequence of mutation in p53, cancer cells escape apoptosis, DNA damage is not repaired, the cell cycle is not halted at the checkpoints to repair damaged DNA, and therefore cells may survive and proliferate with a mutated genome.

A common result is that the chromosomes become increasingly fragmented and incorrectly rejoin, created through successive rounds of cell division of an increasingly mutated genome.

The accumulative loss of tumour suppressor genes and mutation of proto-oncogenes to form oncogenes results in enhanced cell proliferation. In addition, gene amplification, which, in turn, may cause the acquisition of tumour drug resistance. Collectively these changes result in a significant proliferative advantage to the cell.

p53 and consequences for cancer treatment

If we could reactivate p53 function in cancer cells this could potentially be of tremendous therapeutic value as it would induce cancer cell death.

Several recent studies have utilised small peptide molecules that restore function to p53, which have shown tremendous promise.

Cellular functions of p53 (cont)

p53 plays a role in determining sensitivity to radiotherapy. Tumour cells frequently acquire mutations, which decreases the tumour sensitivity to radiation. As radiation induces DNA damage, one of the critical molecules that mediate sensitivity to radiation is p53, therefore understanding p53 function may help scientists and clinicians improve responses in cancer patients to radiotherapy and minimise radiation resistance.

Radiation can kill tumour cells, but the dose that is given is limited by the dose that can be tolerated by the surrounding normal tissues. DNA damage is the main damage induced by radiation to the cell, which in turn, induces p53 immediate responses leading to apoptosis.

Cellular response to radiation therapy is tissue specific, i.e., that is cells, which are rapidly growing tend to apoptose, while fibroblast type tissues, the structural components of organs, tend to undergo growth arrest. Normal cells exposed to radiation will apoptose via p53-mediated pathways.

Tumours are generally very sensitive to radiation because of loss of negative growth control, however, tumours do not in general apoptose in response to radiotherapy as many have lost p53 function. The anti-tumour effect of radiotherapy is mediated by mitotic catastrophe, or irreversible growth arrest. The radiation therapy outcome can be affected by the DNA damage response to both normal and damage cells, so p53 function can reduce normal tissue damage and sensitise tumour cells for treatment.

Ionizing radiation on normal cells and cancer