CANCER

What Is Cancer?

Clinically, cancer is defined as a large number of complex diseases, up to a hundred, that behave differently depending on the cell types from which they originate. Cancers vary in their ages of onset, growth rates, invasiveness, prognoses, and responsiveness to treatments. However, at the molecular level, all cancers exhibit common characteristics that unite them as a family.

Cancer cells are defined by two heritable properties:

(1) They reproduce in defiance of the normal restraints on cell growth and division (**proliferation**), and
(2) They invade and colonize territories normally reserved for other cells (**metastasis**).

It is the combination of these properties that makes cancers particularly dangerous. In normal cells, these functions are tightly controlled by genes that are expressed appropriately in time and place. In cancer cells, these genes are either mutated or are expressed inappropriately. An abnormal cell that grows (increases in mass) and proliferates (divides) out of control will give rise to a **tumor**, or ***neoplasm***—literally, a new growth. As long as the neoplastic cells have not yet become invasive, however, the tumor is said to be **benign**. For most types of such neoplasms, removing or destroying the mass locally usually achieves a complete cure. A tumor is considered a true cancer if it is **malignant**; that is, when its cells have acquired the ability to invade surrounding tissue. Invasiveness is an essential characteristic of cancer cells. It allows them to break loose, enter blood or lymphatic vessels, and form secondary tumors called **metastases** at other sites in the body. In general, the more widely a cancer spreads, the harder it becomes to eradicate. It is generally metastases that kill the cancer patient.

Cancers are traditionally classified according to the tissue and cell type from which they arise. **Carcinomas** are cancers arising from epithelial cells, and they are by far the most common cancers in humans. They account for about 80% of cases, perhaps because most of the cell proliferation in adults occurs in epithelia. In addition, epithelial tissues are the most likely to be exposed to the various forms of physical and chemical damage that favor the development of cancer. **Sarcomas** arise from connective tissue or muscle cells. Cancers that do not fit in either of these two broad categories include the various **leukemias** and **lymphomas**, derived

from white blood cells and their precursors (hemopoietic cells), as well as cancers derived from cells of the nervous system.

In parallel with the set of names for malignant tumors, there is a related set of names for benign tumors: an ***adenoma***, for example, is a benign epithelial tumor with a glandular organization; the corresponding type of malignant tumor is an ***adenocarcinoma***. Similarly, a ***chondroma*** and a ***chondrosarcoma*** are, respectively, benign and malignant tumors of cartilage. Most cancers have characteristics that reflect their origin. Thus, for example, the cells of a *basal-cell carcinoma*, derived from a keratinocyte stem cell in the skin, generally continue to synthesize cytokeratin intermediate filaments, whereas the cells of a ***melanoma***, derived from a pigment cell in the skin, will often (but not always) continue to make pigment granules. Cancers originating from different cell types are, in general, very different diseases. Basal-cell carcinomas of the skin, for example, are only locally invasive and rarely metastasize, whereas melanomas can become much more malignant and often form metastases. Basal-cell carcinomas are readily cured by surgery or local irradiation, whereas malignant melanomas, once they have metastasized widely, are usually fatal.

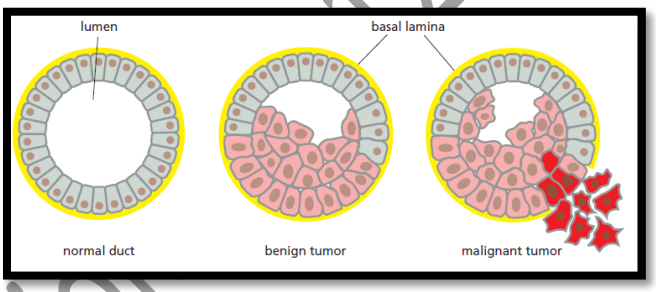

Fig: Benign versus malignant tumors. A benign glandular tumor (*pink* cells; an adenoma) remains inside the basal lamina *(yellow)* that marks the boundary of the normal structure (a duct, in this example). In contrast, a malignant glandular tumor (*red* cells; an adenocarcinoma) can develop from a benign tumor cell, and it destroys the integrity of the tissue, as shown. There are many different forms that such tumors may take.

The Clonal Origin of Cancer Cells

Although malignant tumors may contain billions of cells, and may invade and grow in numerous parts of the body, all cancer cells in the primary and secondary tumors are clonal, meaning that they originated from a common ancestral cell that accumulated specific mutations. This is an important concept in understanding the molecular causes of cancer and has implications for its diagnosis. Numerous data support the concept of cancer clonality.

One way of proving clonal origin is through molecular analysis of the chromosomes in tumor cells. In almost all patients with ***chronic myelogenous leukemia*** (***CML***), for example, we can distinguish the leukemic white blood cells from the patient's normal cells by a specific chromosomal abnormality: the so-called ***Philadelphia chromosome***, created by a translocation between the long arms of chromosomes 9 and 22. When the DNA at the site of translocation is cloned and sequenced, it is found that the site of breakage and rejoining of the translocated fragments is identical in all the leukemic cells in any given patient, but that this site differs slightly (by a few hundred or thousand base pairs) from one patient to another. This is the expected result if, and only if, the cancer in each patient arises from a unique accident occurring in a single cell.

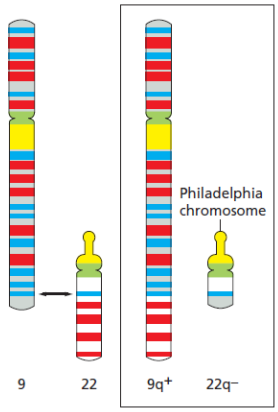

Fig: The translocation between chromosomes 9 and 22 responsible for chronic myelogenous leukemia.

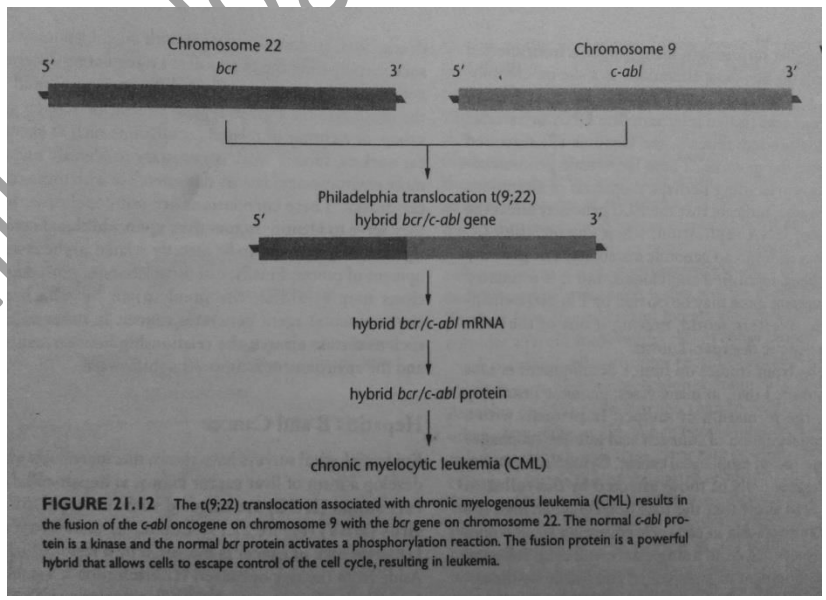

FIGURE 21.12 The t(9;22) translocation associated with chronic myelogenous leukemia (CML) results in the fusion of the *c-abl* oncogene on chromosome 9 with the *bcr* gene on chromosome 22. The normal *c-abl* protein is a kinase and the normal *bcr* protein activates a phosphorylation reaction. The fusion protein is a powerful hybrid that allows cells to escape control of the cell cycle, resulting in leukemia.

Another example, Cancer cells from patients with **Burkitt's lymphoma** show reciprocal translocations between chromosome 8 (with translocation breakpoints at or near the *c-myc* gene) and chromosomes 2, 14, or 22 (with translocation breakpoints at or near one of the immunoglobulin genes). Each Burkitt's lymphoma patient exhibits unique breakpoints in his or her *c-myc* and immunoglobulin gene DNA sequences; however, all lymphoma cells within that patient contain identical translocation breakpoints. This demonstrates that all cancer cells in each case of Burkitt's lymphoma arise from a single cell, and this cell passes on its genetic aberrations to its progeny.

The Cancer Stem Cell Hypothesis

A concept that is related to the clonal origin of cancer cells is that of the cancer stem cell. Many scientists now believe that tumors are comprised of a mixture of cells, many of which do not proliferate. Those that do proliferate and give rise to all the cells within the tumor are known as **cancer stem cells**. Stem cells are cells that have the capacity for self-renewal—a process in which the stem cell divides unevenly, creating one daughter cell that goes on to differentiate into a mature cell type and one that remains a stem cell. The **cancer stem cell hypothesis** contrasts the **random** or **stochastic model**. This model predicts that every cell within a tumor has the potential to form a new tumor. Although scientists still actively debate the existence of cancer stem cells, evidence is accumulating that cancer stem cells do exist, at least in some tumors. Cancer stem cells have been identified in leukemias as well as in solid tumors of the brain, breast, colon, ovary, pancreas, and prostate. It is still not clear what fraction of any tumor is comprised of cancer stem cells. For example, human acute myeloid leukemias contain less than 1 cancer stem cell in 10,000. In contrast, some solid tumors may contain as many as 40 percent cancer stem cells. Scientists are also not sure about the origins of cancer stem cells. It is possible that they may arise from normal adult stem cells within a tissue, or they may be created from more differentiated cells that acquire properties similar to stem cells after accumulating numerous mutations.

Cancer cells contain genetic defects affecting cell cycle regulation:

One of the fundamental aberrations in all cancer cells is a loss of control over cell proliferation. Cell proliferation is the process of cell growth and division that is essential for all development and tissue repair in multicellular organisms. Although some cells, such as epidermal cells of the skin or blood-forming cells in the bone marrow, continue to grow and divide throughout an organism's lifetime, most cells in adult multicellular organisms remain in a nondividing, quiescent, and differentiated state. The growth and differentiation of cells must be strictly regulated; otherwise, the integrity of organs and tissues would be compromised by the presence of inappropriate types and quantities of cells. Normal regulation over cell proliferation involves a large number of gene products that control steps in the **cell cycle**, **programmed cell death**, and the **response of cells to external growth signals**. In cancer cells, many of the genes that control these functions are mutated or aberrantly expressed, leading to uncontrolled cell proliferation.

In early to mid-G1, the cell makes a decision either to enter the next cell cycle or to withdraw from the cell cycle into quiescence. Continuously dividing cells do not exit the cell cycle but proceed through G1, S, G2, and M phases; however, if the cell receives signals to stop growing, it enters the **G0** phase of the cell cycle. During G0, the cell remains metabolically active but does not grow or divide. Most differentiated cells in multicellular organisms can remain in this G0 phase indefinitely. Some, such as neurons, never reenter the cell cycle. In contrast, cancer cells are unable to enter G0, and instead, they continuously cycle. Their *rate* of proliferation is not necessarily any greater than that of normal proliferating cells; however, they are not able to become quiescent at the appropriate time or place. Cells in G0 can often be stimulated to reenter the cell cycle by external growth signals. These signals are delivered to the cell by molecules such as growth factors and hormones that bind to cell-surface receptors, which then relay the signal from the plasma membrane to the cytoplasm. The process of transmitting growth signals from the external environment to the cell nucleus is known as **signal transduction**. Ultimately, signal transduction initiates a program of gene expression that propels the cell out of G0 back into the cell cycle. Cancer cells often have defects in signal transduction pathways. Sometimes, abnormal signal transduction molecules send continuous growth signals to the nucleus even in the absence of external growth signals.

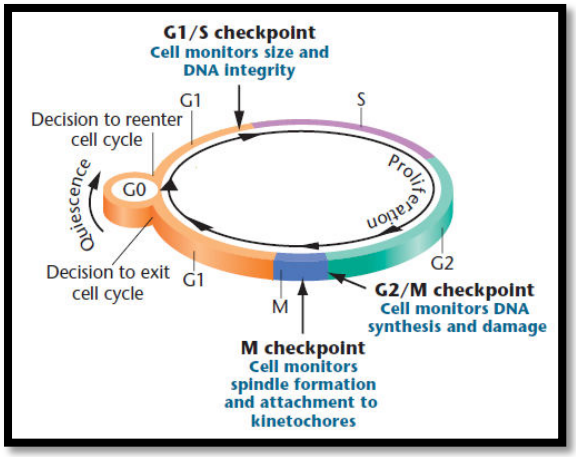

Fig: Checkpoints and proliferation decision points monitor the progress of the cell through the cell cycle.

The cell cycle is regulated by interplay of genes whose products either promote or suppress cell division. Mutation or misexpression of any of the genes controlling the cell cycle can contribute to the development of cancer. For example, if genes that control the G1/S or G2/M checkpoints are mutated, the cell may continue to grow and divide without repairing DNA damage. As these cells continue to divide, they accumulate mutations in genes whose products control cell proliferation or metastasis. Similarly, if genes that control progress through the cell cycle, such as those that encode the cyclins, are expressed at the wrong time or at incorrect levels, the cell may grow and divide continuously and may be unable to exit the cell cycle into G0. The result in both cases is that the cell loses control over proliferation and is on its way to becoming cancerous.

If DNA replication, repair, or chromosome assembly is defective, normal cells halt their progress through the cell cycle until the condition is corrected. This reduces the number of mutations and chromosomal abnormalities that accumulate in normal proliferating cells. However, if DNA or chromosomal damage is so severe that repair is impossible, the cell may initiate a second line of defense—a process called **apoptosis**, or **programmed cell death**. Apoptosis is a genetically controlled process whereby the cell commits suicide. Besides its role in preventing cancer, apoptosis is also initiated during normal multicellular development in order to eliminate certain cells that do not contribute to the final adult organism. A series of proteases called **caspases** are responsible for initiating apoptosis and for digesting intracellular components. Apoptosis is genetically controlled in that regulation of the levels of specific gene products such as Bcl2 and BAX can trigger

or prevent apoptosis. By removing damaged cells, programmed cell death reduces the number of mutations that are passed to the next generation, including those in cancer-causing genes. Some of the same genes that control cell-cycle checkpoints can trigger apoptosis. These genes are mutated in many cancers. As a result of the mutation or inactivation of these checkpoint genes, the cell is unable to repair its DNA or undergo apoptosis. This inability leads to the accumulation of even more mutations in genes that control growth, division, and metastasis.

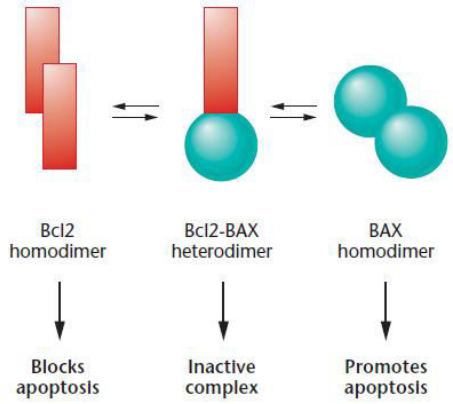

Fig: The relative concentrations of the Bcl2 and BAX proteins regulate apoptosis. A normal cell contains a balance of Bcl2 and BAX, which form inactive heterodimers. A relative excess of Bcl2 results in the formation of Bcl2 homodimers, which prevent apoptosis. Cancer cells with Bcl2 overexpression are resistant to chemotherapies and radiation therapies. A relative excess of BAX results in the formation of BAX homodimers, which induce apoptosis. In normal cells, activated p53 protein induces transcription of the *BAX* gene and inhibits transcription of the *Bcl2* gene, leading to cell death. In many cancer cells, p53 is defective, preventing the apoptotic pathway from removing the cancer cells.

Cancer Cells Display an Altered Control of Growth:

Mutability and large cell population numbers create the opportunities for mutations to occur, but the driving force for development of a cancer has to come from some sort of selective advantage possessed by the mutant cells. Most obviously, a mutation or epigenetic change can confer such an advantage by increasing the rate at which a clone of cells proliferates or by enabling it to continue proliferating when normal cells would stop. Cancer cells that can be grown in culture, or cultured cells artificially engineered to contain the types of mutations encountered in cancers, typically show a transformed phenotype. They are abnormal in their shape, their motility, their responses to growth factors in the culture medium, and, most characteristically, in the way they react to contact with the substratum and

with one another. Normal cells will not divide unless they are attached to the substratum; transformed cells will often divide even if held in suspension. Normal cells become inhibited from moving and dividing when the culture reaches confluence (where the cells are touching one another); transformed cells continue moving and dividing even after confluence, and so pile up in layer upon layer in the culture dish. In addition, transformed cells no longer require all of the positive signals from their surroundings that normal cells require.

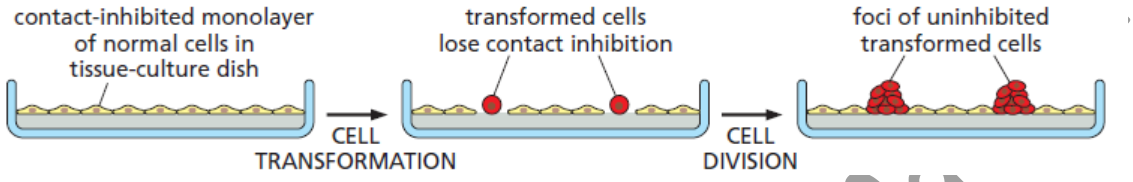

Fig: Loss of contact inhibition by cancer cells in cell culture

Many Properties Typically Contribute to Cancerous Growth:

Clearly, to produce a cancer, a cell must acquire a range of aberrant properties—a collection of subversive new skills—as it evolves. Different cancers require different combinations of these properties. Nevertheless, cancers all share some common features. By definition, they all ignore or misinterpret normal social controls so as to proliferate and spread where normal cells would not. These defining properties are commonly combined with other features that help the miscreants to arise and thrive. A list of the key attributes of cancer cells in general would include the following:

1. They grow (biosynthesize) when they should not, aided by a metabolism shifted from oxidative phosphorylation toward aerobic glycolysis.
2. They go through the cell-division cycle when they should not.
3. They escape from their home tissues (that is, they are invasive) and survive and proliferate in foreign sites (that is, they metastasize).
4. They have abnormal stress responses, enabling them to survive and continue dividing in conditions of stress that would arrest or kill normal cells, and they are less prone than normal cells to commit suicide by apoptosis.
5. They are genetically and epigenetically unstable.
6. They escape replicative cell senescence, either by producing telomerase or by acquiring another way of stabilizing their telomeres.

Proto-oncogenes and Tumor-suppressor Genes Are Altered in Cancer Cells:

All genes whose alteration contributes to the causation or evolution of cancer by driving tumorigenesis are termed as cancer-critical genes

Two general categories of cancer-causing genes are mutated or misexpressed in cancer cells—the **proto-oncogenes** and the **tumor-suppressor genes**.

In general, mitosis can be regulated in two ways:

1) by genes that normally function to suppress cell division, and

2) by genes that normally function to promote cell division.

The first class, called **tumor suppressor genes** (in which a **loss-of-function mutation** can contribute to cancer), inactivates or represses passage through the cell cycle and the resulting cell division. These genes and/or their gene products must be absent or inactive for cell division to take place. If these genes become permanently inactivated or lost through mutation, control over cell division is lost, and the cell begins to proliferate in an uncontrollable fashion.

Genes of the second class, called, **proto-oncogenes** (in which a **gain-of-function mutation** can drive a cell toward cancer), normally function to promote cell division. These genes can be "off" or "on", and when they are on, they promote cell division. To halt cell division, these genes and/or their products must be inactivated. If proto-oncogenes are permanently switched on, then uncontrolled cell division occurs, leading to tumor formation. The mutant, overactive or overexpressed forms of proto-oncogenes are known as **oncogenes.**

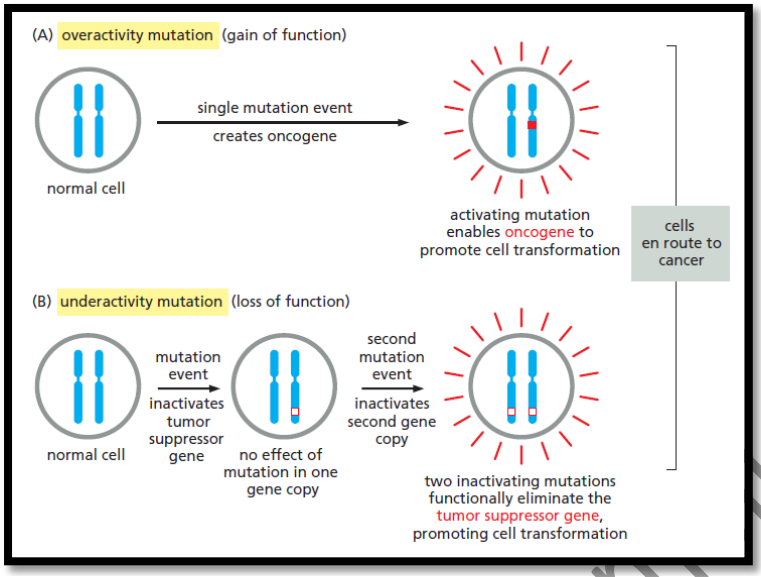

Fig: Cancer-critical mutations fall into two readily distinguishable categories, dominant and recessive

Proto-oncogenes encode **transcription factors** that stimulate expression of other genes, **signal transduction molecules** that stimulate cell division, and **cell-cycle regulators** that move the cell through the cell cycle. Their products are important for normal cell functions, especially cell growth and division. When normal cells become quiescent and cease division, they repress the expression of most proto-oncogenes or modify the activities of their products. In cancer cells, one or more proto-oncogenes are altered in such a way that the activities of their products cannot be regulated in a normal fashion. This is sometimes due to mutations that result in an abnormal protein product. In other cases, proto-oncogenes may be overexpressed or expressed at an incorrect time. If a proto-oncogene is continually in an "on" state, its product may constantly stimulate the cell to divide. When a proto-oncogene is mutated or abnormally expressed and contributes to the development of cancer, it is known as an **oncogene**—a cancer-causing gene. **Oncogenes** are **proto-oncogenes** that have experienced a gain-of-function alteration. As a result, only one allele of a proto-oncogene needs to be mutated or misexpressed in order to contribute to cancer. Hence, oncogenes confer a dominant cancer phenotype.

TABLE 21.6 Cellular Location of *c-onc* and *v-onc* Proteins

Gene	Location of *c-onc* Protein	Location of *v-onc* Protein
src	Membranes	Membranes
ras	Membranes	Membranes
myc	Nucleus	Nucleus
fps	Cytoplasm	Cytoplasm and membranes
abl	Nucleus	Cytoplasm
erbB	Plasma membrane	Plasma membrane and Golgi

At least three mechanisms can explain how proto-oncogenes are converted into oncogenes. These include: **point mutations**, **translocations**, and **overexpression**.

TABLE 21.5 Conversion of Proto-Oncogenes to Oncogenes

Mechanism	Example
Point mutation	*ras*
Translocation	*abl*
Overexpression of gene product	
New promoter by viral insertion	*mos*, *myb*
New enhancer by viral insertion	*myc*
Amplification of proto-oncogene	*myc*

1) A small change in DNA sequence such as a point mutation or deletion may produce a hyperactive protein when it occurs within a protein-coding sequence, or lead to protein overproduction when it occurs withina regulatory region for that gene.
2) Gene amplification events, such as those that can be caused by errors in DNA replication, may produce extra gene copies; this can lead to overproduction of the protein.
3) A chromosomal rearrangement— involving the breakage and rejoining of the DNA helix—may either change the protein-coding region, resulting in a hyperactive fusion protein, or alter the control regions for a gene so that a normal protein is overproduced.

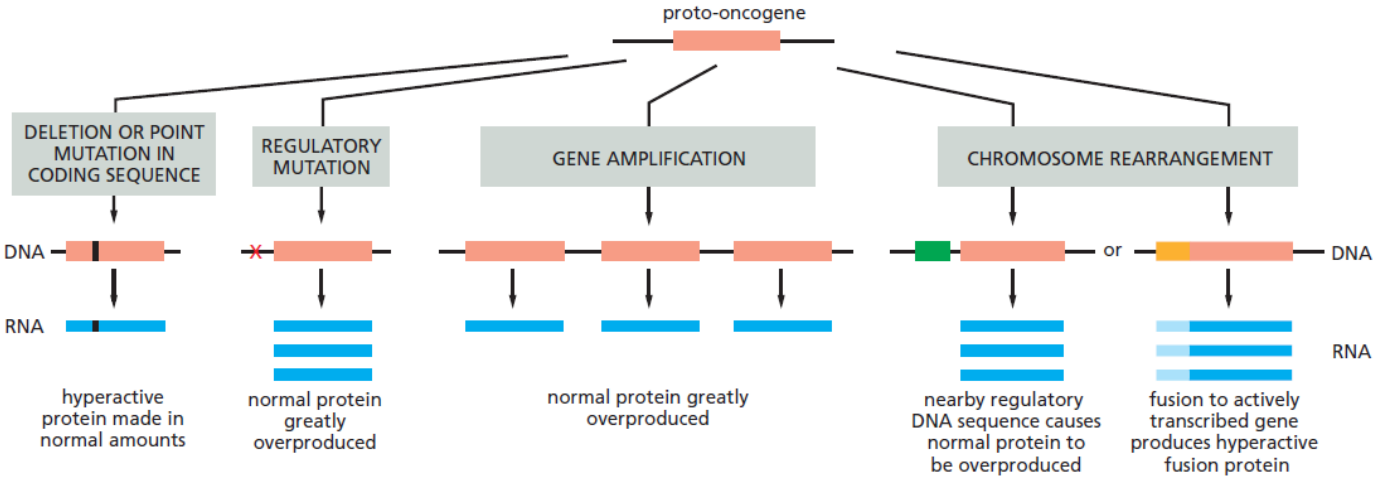

Fig: The types of accidents that can convert a proto-oncogene into an oncogene.

Tumor-suppressor genes are genes whose products normally **regulate cell-cycle checkpoints** or **initiate the process of apoptosis**. In normal cells, proteins encoded by tumor-suppressor genes halt progress through the cell cycle in response to DNA damage or growth-suppression signals from the extracellular environment. When tumor suppressor genes are mutated or inactivated, cells are unable to respond normally to cell-cycle checkpoints, or are unable to undergo programmed cell death if DNA damage is extensive. This leads to the accumulation of more mutations and the development of cancer. When both alleles of a tumor-suppressor gene are inactivated, and other changes in the cell keep it growing and dividing, cells may become **tumorigenic**.

Approximately 400 oncogenes and tumor suppressor genes are now known, and more will likely be discovered as cancer research continues.

Some Proto-oncogenes and Tumor-suppressor Genes

Proto-oncogene	Normal Function	Alteration in Cancer	Associated Cancers
c-myc	Transcription factor, regulates cell cycle, differentiation, apoptosis	Translocation, amplification, point mutations	Lymphomas, leukemias, lung cancer, many types
c-kit	Tyrosine kinase, signal transduction	Mutation	Sarcomas
RARα	Hormone-dependent transcription factor, differentiation	Chromosomal translocations with PML gene, fusion product	Acute promyelocytic leukemia
E6	Human papillomavirus encoded oncogene, inactivates p53	HPV infection	Cervical cancer
Cyclins	Bind to CDKs, regulate cell cycle	Gene amplification, overexpression	Lung, esophagus, many types
Tumor Suppressor	**Normal Function**	**Alteration in Cancer**	**Associated Cancers**
RB1	Cell-cycle checkpoints, binds E2F	Mutation, deletion, inactivation by viral oncogene products	Retinoblastoma, osteosarcoma, many types
APC	Cell–cell interaction	Mutation	Colorectal cancers, brain, thyroid
p53	Transcription regulation	Mutation, deletion, viruses	Many types
BRCA1, BRCA2	DNA repair	Point mutations	Breast, ovarian, prostate cancers

The *ras* Proto-oncogenes :

Some of the most frequently mutated genes in human tumors are those in the ***ras* gene family**. These genes are mutated in more than 30 percent of human tumors. The *ras* gene family encodes a 189 amino acid protein signal transduction molecules that are associated with the cell membrane and regulate cell growth and division. Ras proteins normally transmit signals from the cell membrane to the nucleus, stimulating the cell to divide in response to external growth factors. Ras proteins alternate between an inactive (switched off) and an active (switched on) state by binding either guanosine diphosphate (GDP) or guanosine triphosphate (GTP). When a cell encounters a growth factor (such as platelet-derived growth factor or epidermal growth factor), growth factor receptors on the cell membrane bind to the growth factor, resulting in autophosphorylation of the cytoplasmic portion of the growth factor receptor. This causes recruitment of proteins known as nucleotide exchange factors to the plasma membrane.

These nucleotide exchange factors cause Ras to release GDP and bind GTP, thereby activating Ras. The active, GTP-bound form of Ras then sends its signals through cascades of protein phosphorylations in the cytoplasm. The end-point of these cascades is activation of nuclear transcription factors that stimulate expression of genes whose products drive the cell from quiescence into the cell cycle. Once Ras has sent its signals to the nucleus, it hydrolyzes GTP to GDP and becomes inactive.

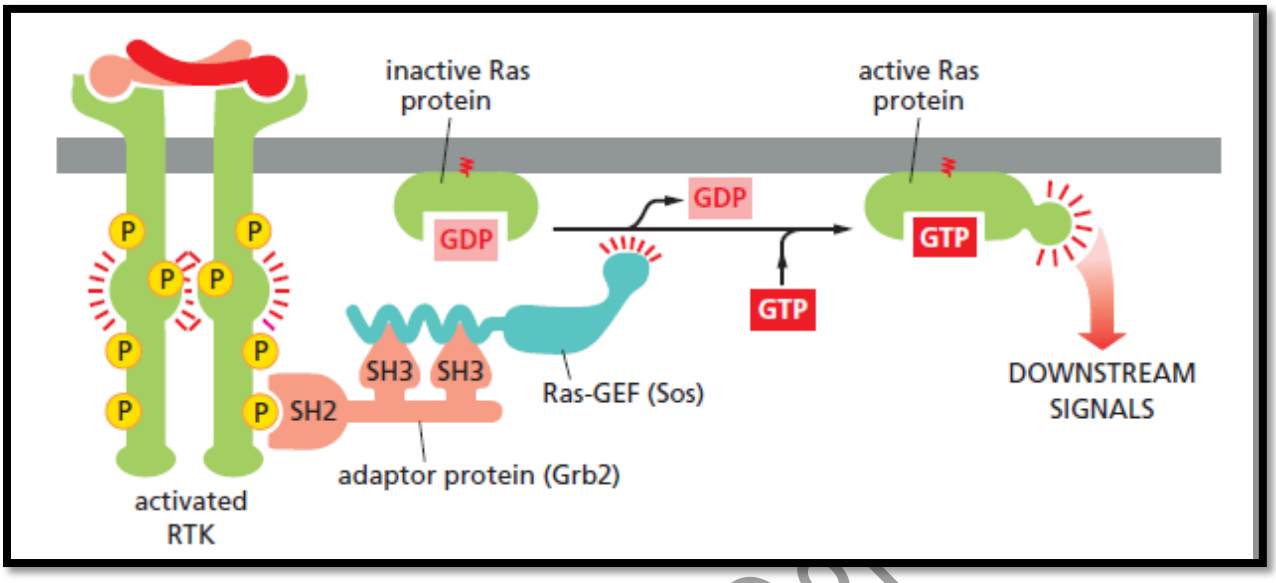

Fig: RTK activates Ras

Mutations that convert the *ras* proto-oncogene to an oncogene prevent the Ras protein from hydrolyzing GTP to GDP and hence freeze the Ras protein into its "on" conformation, constantly stimulating the cell to divide. Comparison of the amino acid sequence of Ras proteins from a number of different human carcinomas reveals that ras mutations involve single amino acid substitutions at either position 12 or 61.

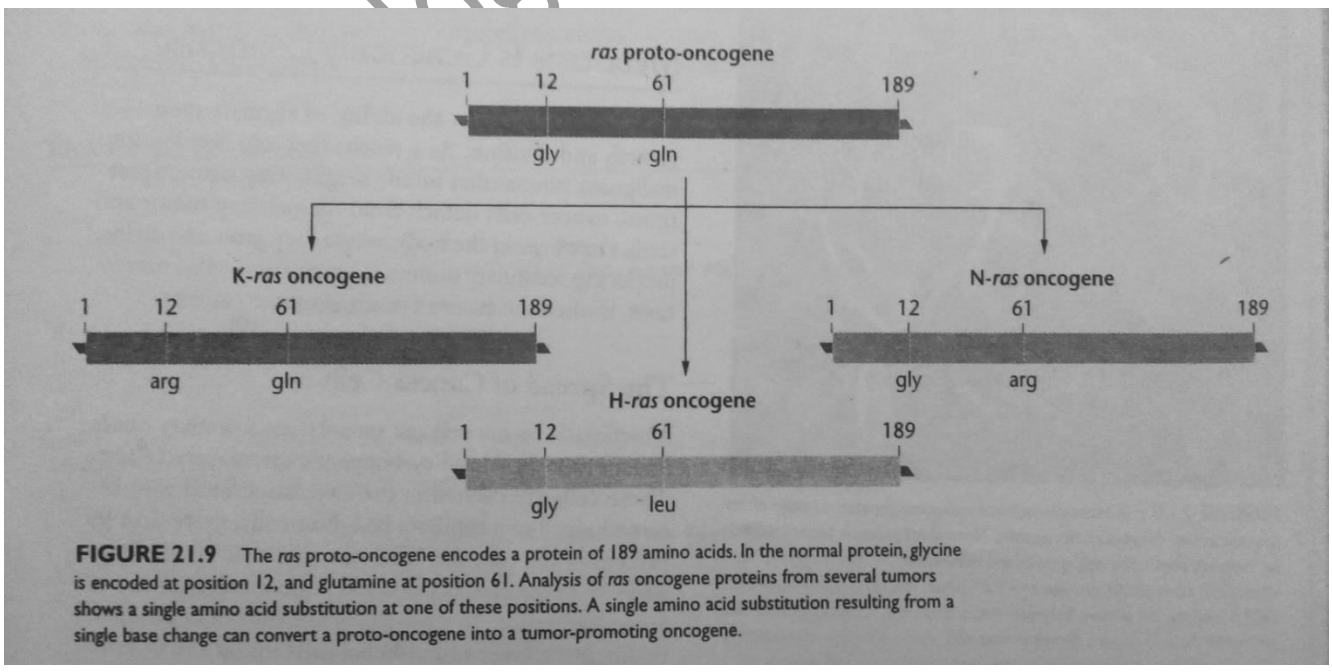

FIGURE 21.9 The *ras* proto-oncogene encodes a protein of 189 amino acids. In the normal protein, glycine is encoded at position 12, and glutamine at position 61. Analysis of *ras* oncogene proteins from several tumors shows a single amino acid substitution at one of these positions. A single amino acid substitution resulting from a single base change can convert a proto-oncogene into a tumor-promoting oncogene.

The *RB1* Tumor-suppressor Gene / Retinoblastoma:

The discovery of the first tumor suppressor gene ***RB1*** **(retinoblastoma 1**) came from studies of a rare type of human cancer, **retinoblastoma**, which arises from cells in the retina of the eye that are converted to a cancerous state by an unusually small number of mutations. Retinoblastoma occurs in childhood, and tumors develop from neural precursor cells in the immature retina. Retinoblastoma occurs with a frequency of about 1 in 15,000-20000 individuals. One form of the disease is hereditary, and the other is not. In the hereditary (**familial**) form of the disease, individuals inherit one mutated allele of the *RB1* gene and have an 85 percent chance of developing retinoblastomas as well as an increased chance of developing other cancers. All somatic cells of patients with hereditary retinoblastoma contain one mutated allele of the *RB1* gene. However, it is only when the second normal allele of the *RB1* gene is lost or mutated in certain retinal cells that retinoblastoma develops. Multiple tumors usually arise independently, affecting both eyes. In the nonhereditary form, retinoblastoma is extremely rare, as it requires at least two separate somatic mutations in a retinal cell in order to inactivate both copies of the *RB1* gene, only one eye is affected, and by only one tumor. A few individuals with retinoblastoma have a visibly abnormal karyotype, with a deletion of a specific band on **chromosome 13** that, if inherited, predisposes an individual to the disease. Deletions of this same region are also encountered in tumor cells from some patients with the nonhereditary disease, which suggested that the cancer was caused by loss of a critical gene in that location.

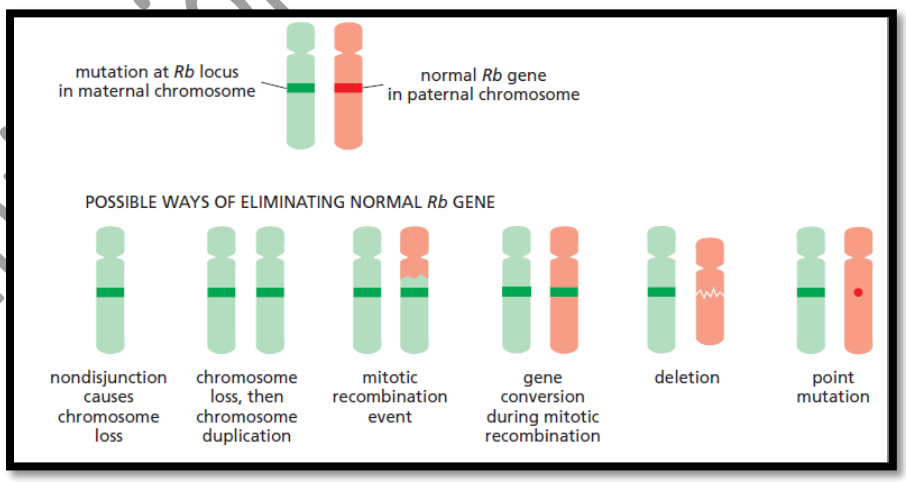

Fig: Six ways of losing the remaining good copy of a tumor suppressor gene through a change in DNA sequences.

The **retinoblastoma protein (pRB)** is a tumor-suppressor protein that controls the G1/S cell-cycle checkpoint. The pRB protein is found in the nuclei of all cell types at all stages of the cell cycle. However, its activity varies throughout the cell cycle, depending on its phosphorylation state. When cells are in the G0 phase of the cell cycle, the pRB protein is nonphosphorylated and binds to transcription factors such as E2F, inactivating them. When the cell is stimulated by growth factors, it enters G1 and approaches S phase. Throughout the G1 phase, the pRB protein becomes phosphorylated by the CDK4/cyclin D1 complex. Phosphorylated pRB releases its bound regulatory proteins. When E2F and other regulators are released by pRB, they are free to induce the expression of over 30 genes whose products are required for the transition from G1 into S phase. After cells traverse S, G2, and M phases, pRB reverts to a nonphosphorylated state, binds to regulatory proteins such as E2F, and keeps them sequestered until required for the next cell cycle. In normal quiescent cells, the presence of the pRB protein prevents passage into S phase. In many cancer cells, including retinoblastoma cells, both copies of the *RB1* gene are defective, inactive, or absent, and progression through the cell cycle is not regulated.

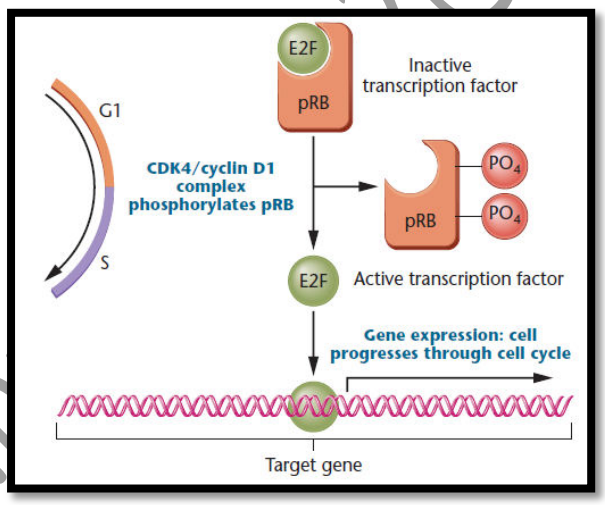

Fig: During G0 and early G1, pRB interacts with and inactivates transcription factor E2F. As the cell moves from G1 to S phase, a CDK4/cyclinD1 complex forms and adds phosphate groups to pRB. As pRB becomes phosphorylated, E2F is released and becomes transcriptionally active, allowing the cell to pass through S phase. Phosphorylation of pRB is transitory; as CDK/cyclin complexes are degraded and the cell moves through the cell cycle to early G1, pRB phosphorylation declines, allowing pRB to reassociate with E2F.

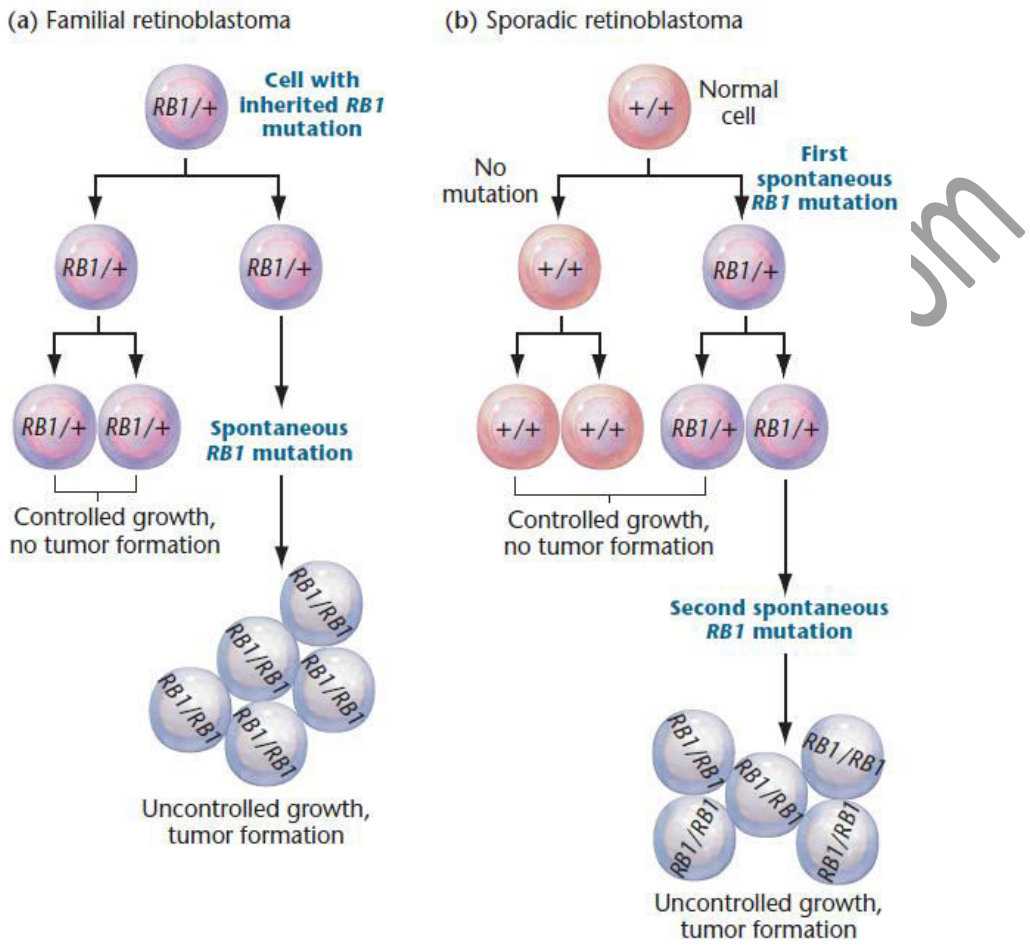

Fig: (a) In familial retinoblastoma, one mutation (designated as *RB1*) is inherited and present in all cells. A second mutation at the retinoblastoma locus in any retinal cell contributes to uncontrolled cell growth and tumor formation. (b) In sporadic retinoblastoma, independent mutations in both alleles of the retinoblastoma gene within a single cell are acquired sequentially, also leading to tumor formation.

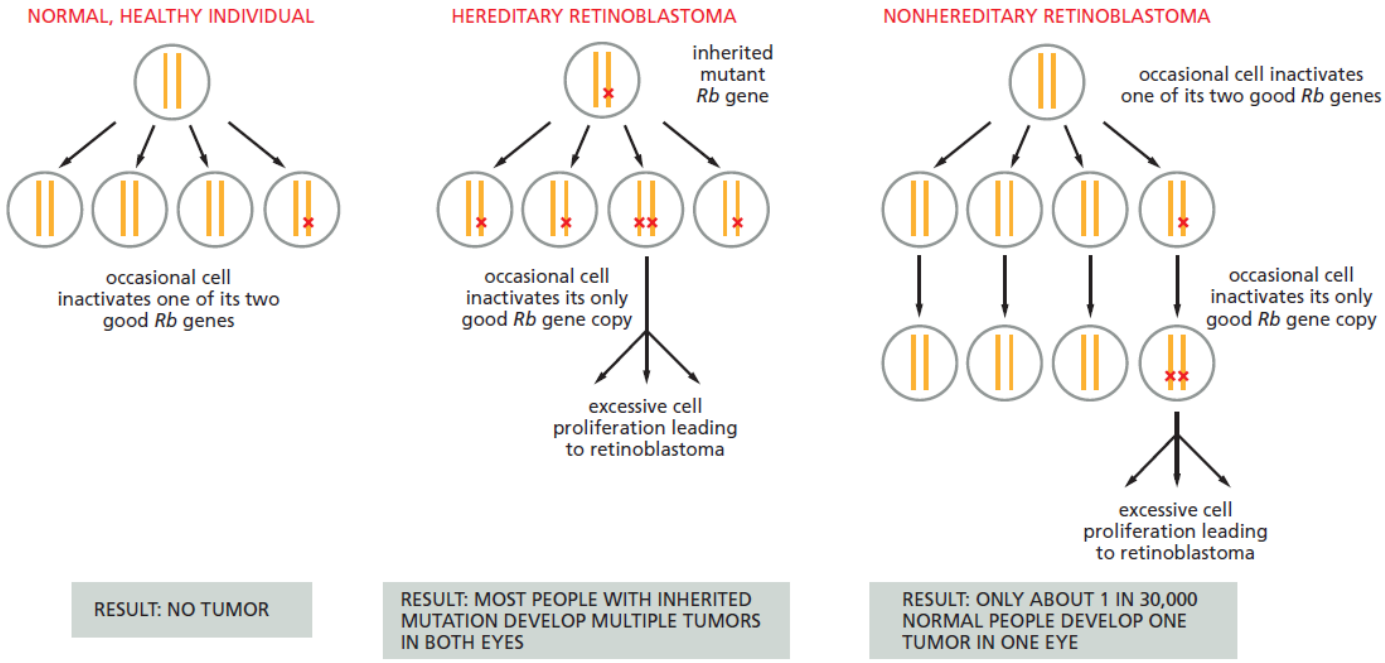

Fig: The genetic mechanisms that cause retinoblastoma. In the hereditary form, all cells in the body lack one of the normal two functional copies of the *Rb* tumor suppressor gene, and tumors occur where the remaining copy is lost or inactivated by a somatic event (either mutation or epigenetic silencing). In the nonhereditary form, all cells initially contain two functional copies of the gene, and the tumor arises because both copies are lost or inactivated through the coincidence of two somatic events in a single line of cells.

Knudson Model:

By studying the two different forms of retinoblastoma, Alfred Knudson and his colleagues developed a model that requires the presence of two mutated copies of the Rb gene in the same retinal cell for tumor development.(*i.e.,* the mutant allele is recessive).This model explains how the mutation itself can act asa recessive trait, and how carrying one mutant allele acts as a dominant trait in predisposing an individual to cancer (they need only one additional mutation)

The *p53* Tumor-suppressor Gene:

The most frequently mutated gene in human cancers— mutated in more than 50 percent of all cancers —is the ***p53* gene**. This gene encodes a nuclear protein that acts as a transcription factor, repressing or stimulating transcription of more than 50 different genes.

Most cells in the body have very little p53 protein under normal conditions: although the protein is synthesized, it is rapidly degraded. Moreover, p53 is not essential for normal development. The p53 pathway, therefore, behaves as a sort of antenna, sensing the presence of a wide range of dangerous conditions, and when any are detected, triggering appropriate action—either a temporary or permanent arrest of cell cycling (senescence), or suicide by apoptosis. These responses serve to prevent deranged cells from proliferating. Cancer cells are indeed generally deranged, and their survival and proliferation thus depend on inactivation of the p53 pathway.

The p53 protein performs its job mainly by acting as a transcription regulator. Indeed, the most common mutations observed in p53 in human tumors are in its DNA-binding domain, where they cripple the ability of p53 to bind to its DNA target sequences. Because p53 binds to DNA as a tetramer, a single mutant subunit within a tetrameric complex can be enough to block its function. Thus, mutations in *p53* can have a dominant negative effect, causing loss of p53 function even when the cell also contains a wild-type version of the gene. For this reason, in contrast with other tumor suppressor genes such as *Rb*, the development of cancer does not always require that both copies of *p53* be knocked out.

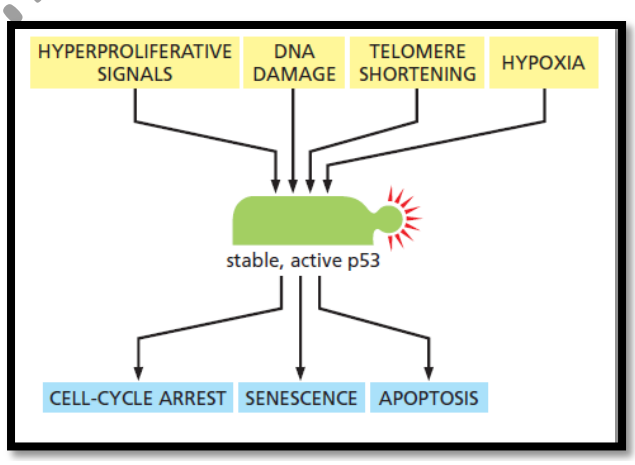

Fig: Modes of action of the p53 tumor suppressor

Normally, the p53 protein is continuously synthesized but is rapidly degraded and therefore is present in cells at low levels. In addition, the p53 protein is normally bound to another protein called **MDM2**, which has several effects on p53. The presence of MDM2 on the p53 protein tags p53 for degradation and sequesters the transcriptional activation domain of p53. It also prevents the **phosphorylations** and **acetylations** that convert the p53 protein from an inactive to an active form. Several types of cellular stress events bring about rapid increases in the nuclear levels of activated p53 protein. These include chemical damage to DNA, double-stranded breaks in DNA induced by ionizing radiation, and the presence of DNA-repair intermediates generated by exposure of cells to ultraviolet light. In response to these signals, MDM2 dissociates from p53, making p53 more stable and unmasking its transcription activation domain. Increases in the levels of activated p53 protein also result from increases in protein phosphorylation, acetylation, and other post-translational modifications. Activated p53 protein acts as a transcription factor that stimulates expression of the *MDM2* gene. As the levels of MDM2 increase, p53 protein is again bound by MDM2, returned to an inactive state, and targeted for degradation, in a negative feedback loop.

The p53 protein initiates several different responses to DNA damage including cell-cycle arrest followed by DNA repair and apoptosis if DNA cannot be repaired. These responses are accomplished by p53 acting as a transcription factor that stimulates or represses the expression of genes involved in each response.

In normal cells, p53 can arrest the cell cycle at the G1/S and G2/M checkpoints, as well as retarding the progression of the cell through S phase. To arrest the cell cycle at the G1/S checkpoint, activated p53 protein stimulates transcription of a gene encoding the p21 protein. The p21 protein inhibits the CDK4/cyclin D1 complex, hence preventing the cell from moving from G1 phase into S phase. Activated p53 protein also regulates expression of genes that retard the progress of DNA replication, thus allowing time for DNA damage to be repaired during S phase. By regulating expression of other genes, activated p53 can block cells at the G2/M checkpoint, if DNA damage occurs during S phase.

Activated p53 can also instruct a damaged cell to commit suicide by apoptosis. It does so by activating the transcription of the *Bax* gene and repressing transcription of the *Bcl2* gene. In normal cells, the BAX protein is present in a heterodimer with the Bcl2 protein, and the cell remains viable (Figure 19–8). But when the levels of BAX protein increase in response to p53 stimulation of *Bax* gene transcription, BAX homodimers are formed, and these homodimers activate the cellular changes that lead to apoptosis. In cancer cells that lack functional p53,

BAX protein levels do not increase in response to cell damage, and apoptosis may not occur.

Cells lacking functional p53 are unable to arrest at cell cycle checkpoints or to enter apoptosis in response to DNA damage. As a result, they move unchecked through the cell cycle, regardless of the condition of the cell's DNA. Cells lacking p53 have high mutation rates and accumulate the types of mutations that lead to cancer. Because of the importance of the *p53* gene to genomic integrity, it is often referred to as the "guardian of the genome."

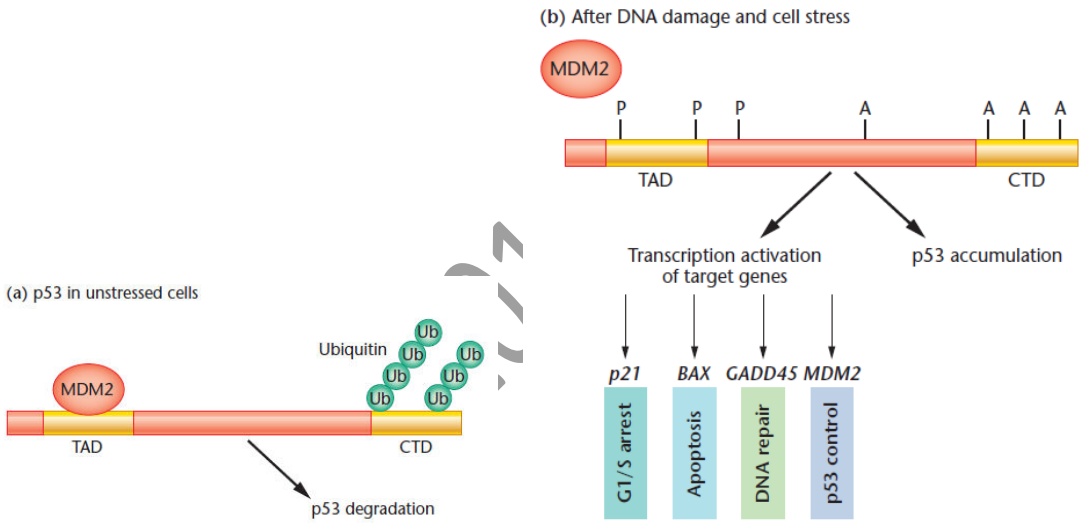

Fig: Steps in the regulation of p53 levels and activity. (a) In normal unstressed cells, p53 is kept inactive and at low abundance by MDM2, which binds to the transactivation domain (TAD) and stimulates the addition of ubiquitin onto lysine residues in the carboxy-terminal domain (CTD). The presence of ubiquitin promotes p53 degradation. (b) After various types of cellular stress including DNA damage, cellular kinases add phosphates (P's) to serines and threonines in the TAD, leading to dissociation of MDM2 and subsequent loss of ubiquitin. As the levels of p53 increase in the nucleus, histone acetyl transferases add acetyl groups (A's) to lysines in the CTD, which increases p53 stability and affinity for specific DNA sequences within the promoter regions of target genes. Examples of genes that are transcriptionally stimulated by p53 are *p21* (leading to G1/S cell cycle arrest), *BAX* (stimulating apoptosis), *GADD45* (contributing to DNA repair), and *MDM2* (returning p53 to an inactive and low abundance state).

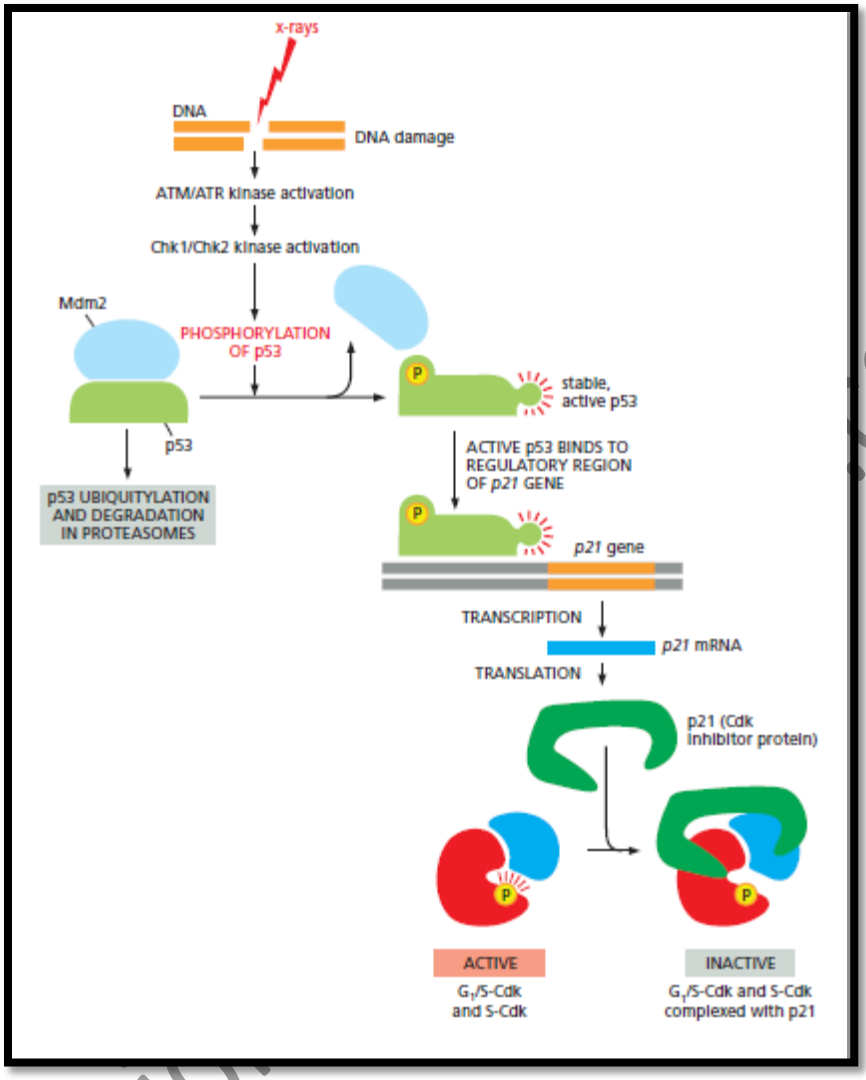

Fig: How DNA damage arrests the cell cycle in G1

A single mutation is not enough to change a normal cell into a cancer cell indicating that cancer is a multistep process, requiring multiple mutations:

Although we know that cancer is a genetic disease initiated by mutations that lead to uncontrolled cell proliferation and metastasis, a single mutation is not sufficient to transform a normal cell into a tumor-forming (tumorigenic), malignant cell. Many lines of evidence indicate that the development of a cancer typically requires that a substantial number of independent, rare genetic and epigenetic accidents occur in the lineage that emanates from a single cell.

1) In humans, mutations occur spontaneously at a rate of about 10- 6 mutations per gene, per cell division, mainly due to the intrinsic error rates of DNA replication. Because there are approximately 10^{16} cell divisions in a human body during a lifetime, a person might suffer up to 10^{10} mutations per gene somewhere in the body, during his or her lifetime. However, only about one person in three will suffer from cancer which would be far more prevalent than it is.
2) The phenomenon of age-related cancer is another indication that cancer develops from the accumulation of several mutagenic events in a single cell. The incidence of most cancers rises exponentially with age. If a single mutation were sufficient to convert a normal cell to a malignant one, then cancer incidence would appear to be independent of age. The age-related incidence of cancer suggests that many independent mutations, occurring randomly, and with a low probability, are necessary before a cell is transformed into a malignant cancer cell.

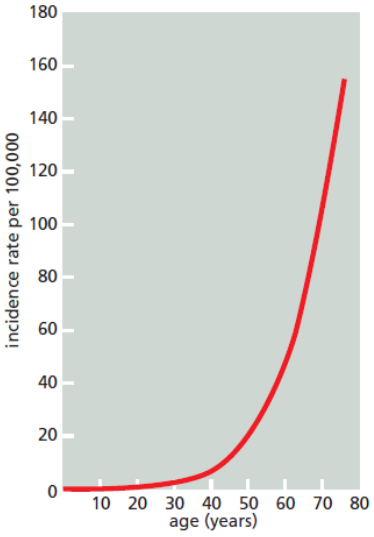

Fig: Cancer incidence as a function of age

3) Another indication that cancer is a multistep process is the delay that occurs between exposure to **carcinogens** (cancer-causing agents) and the appearance of the cancer. For example, an incubation period of five to eight years separated exposure of people to the radiation of the atomic explosions at Hiroshima and Nagasaki and the onset of leukemias.

The **multistep nature of cancer development** is supported by the observation that it requires a gradual accumulation of mutations in a number of different genes, helps to explain the well-known phenomenon of tumor progression, whereby an initial mild disorder of cell behavior evolves gradually into a full-blown cancer. This progressive nature of cancer is illustrated by the development of **colon cancer**. Each step in **tumorigenesis** (the development of a malignant tumor) appears to be the result of two or more genetic alterations that release the cells progressively from the controls that normally operate on proliferation and malignancy. Also **Chronic myelogenous leukemia** provides a clear example. It begins as a disorder characterized by a nonlethal overproduction of white blood cells and continues in this form for several years before changing into a much more rapidly progressing illness that usually ends in death within a few months. In the early chronic phase, the leukemic cells are distinguished mainly by the chromosomal translocation (the Philadelphia chromosome) mentioned previously, although there may well be other, less visible genetic or epigenetic changes. In the subsequent acute phase, cells that show not only the translocation but also several other chromosomal abnormalities overrun the hemopoietic (blood-forming) system. It appears that cells from the initial mutant clone have undergone further mutations that make them proliferate even more vigorously, so that they come to outnumber both the normal blood cells and their ancestors with the primary chromosomal translocation.

This observation suggests that the progressive genetic alterations that create a cancer cell confer selective advantages to the cell and are propagated through cell divisions during the creation of tumors.

APC:

The development of hereditary colon cancer illustrates how inherited mutations in one allele of a gene contribute only one step in the multistep pathway leading to malignancy. About 1 percent of colon cancer cases result from a genetic predisposition to cancer known as **familial adenomatous polyposis (FAP)**. In FAP, individuals inherit one mutant copy of the ***APC*** **(adenomatous polyposis) gene** located on the long arm of **chromosome 5**. Mutations include deletions, frameshift, and point mutations. The normal function of the *APC* gene product is to act as a tumor suppressor controlling cell–cell contact and growth inhibition by interacting with the β-catenin protein. The presence of a heterozygous *APC* mutation causes the epithelial cells of the colon to partially escape cell-cycle control, and the cells divide to form small clusters of cells called **polyps** or

adenomas. People who are heterozygous for this condition develop hundreds to thousands of colon and rectal polyps early in life. Although it is not necessary for the second allele of the *APC* gene to be mutated in polyps at this stage, in the majority of cases, the second *APC* allele becomes mutant in a later stage of cancer development. The relative order of mutations in the development of colon cancer is shown in Figure. The second mutation in polyp cells that contain an *APC* gene mutation occurs in the *ras* proto-oncogene. The combined *APC* and *ras* gene mutations bring about the development of intermediate adenomas. Cells within these adenomas have defects in normal cell differentiation. In addition, these cells will grow in culture and are not growth inhibited by contact with other cells—a process known as **transformation**. The third step toward malignancy requires loss of function of both alleles of the ***DCC*** (*d*eleted in *c*olon *c*ancer) gene. The *DCC* gene product is thought to be involved with cell adhesion and differentiation. Mutations in both *DCC* alleles result in the formation of late-stage adenomas with a number of finger-like outgrowths (villi). When late adenomas progress to cancerous adenomas, they usually suffer loss of functional *p53* genes. The final steps toward malignancy involve mutations in an unknown number of genes associated with metastasis.

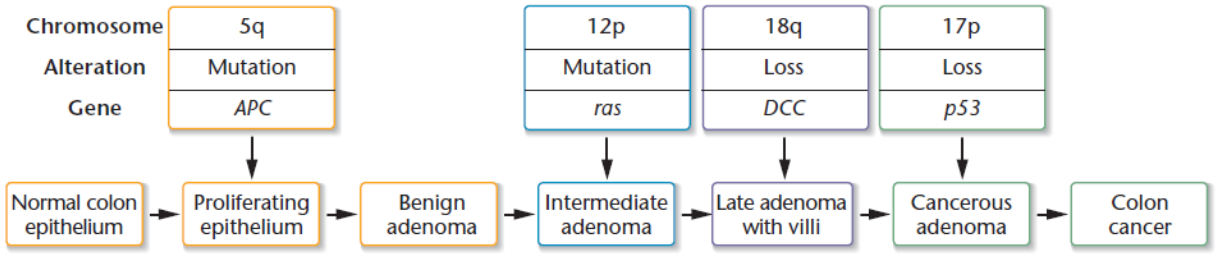

Fig: A model for the multistep development of colon cancer. The first step is the loss or inactivation of one allele of the *APC* gene on chromosome 5. In FAP cases, one mutant *APC* allele is inherited. Subsequent mutations involving genes on chromosomes 12, 17, and 18 in cells of benign adenomas can lead to a malignant transformation that results in colon cancer. Although the mutations on chromosomes 12, 17, and 18 usually occur at a later stage than those involving chromosome 5, the sum of changes is more important than the order in which they occur.