Cancer

WHAT IS CANCER?

Cancer is a disease which occurs when changes in a group of normal cells within the body lead to uncontrolled growth **causing a lump called a tumour**; this is true of all cancers except leukaemia (cancer of the blood).

If left untreated, **tumours can grow and spread into the surrounding normal tissue**, or to other parts of the body via the bloodstream and lymphatic systems, and can affect the digestive, nervous and circulatory systems.

TUMOURS (LUMPS) CAN BE BENIGN OR MALIGNANT

•**Benign tumours** are **not cancerous** and **rarely threaten life**.

- They **tend to grow quite slowly, do not spread to other parts of the body** and are usually made up of cells quite similar to normal / healthy cells.
- They will only cause a problem if they grow very large, becoming uncomfortable or press on other organs for example a brain tumour inside the skull.

- **Malignant tumours** are **faster growing than benign tumours** and have the **ability to spread and destroy neighbouring tissue**.
  - Cells of **malignant tumours can break off from the main (primary) tumour** and **spread to other parts of the body through a process known as metastasis**.
  - Upon **invading healthy tissue** at the **new site they continue to divide and grow.** These secondary sites are known as **metastases** and the condition is referred to as **metastatic cancer**.

CANCER CAN BE CLASSIFIED ACCORDING TO THE FOLLOWING CATEGORIES

Carcinoma

- A cancer that arises from the **epithelial cells** (the lining of cells that helps protect or enclose organs). Carcinomas **may invade the surrounding tissues and organs and metastasise** to the **lymph nodes and other areas of the body**. The most common forms of cancer in this group are **breast, prostate, lung and colon cancer.**

Sarcoma

- A type of **malignant tumour of the bone or soft tissue** (fat, muscle, blood vessels, nerves and other connective tissues that support and surround organs). The most common forms of sarcoma are **leiomyosarcoma, liposarcoma and osteosarcoma**.

Lymphoma

- Lymphoma is a **cancer of the lymphatic system**, which **runs all through the body**, and can **therefore occur anywhere**. The two main forms are **nonHodgkin's** which begins with

- uncontrolled growth of the **-white blood cells -lymphocytes** -of the immune system) and **Hodgkin's lymphoma** in which cells of the **lymph nodes become cancerous**.

Leukaemia

- Leukaemia is a cancer of the **white blood cells and bone marrow**, the tissue that forms blood cells. There are several subtypes; common are **lymphocytic leukaemia** and **chronic lymphocytic leukaemia.**

CAUSES OF CANCER

There are about **200 known types of cancer**.
As with most illnesses cancer is multifactorial, meaning there is no single cause for any one type of cancer.

Cancer-causing substances (carcinogens)

- Genes are coded messages inside a cell that tell it how to behave (i.e. which proteins to make).
- Mutation or changes to the gene, such as damage or loss, can alter how that cell behaves.
- For example, a **mutation may mean that too much protein is made,** or that **protein is not made at all**. Significantly, there needs to be a number of genetic mutations within a cell before it becomes cancerous.
- Something that damages a cell, changing its behaviour and makes it more likely to be cancerous is called a 'carcinogen.

Age

- Many types of cancer become more prevalent with age.
- The **longer people live, the more exposure there is to carcinogens** and the **more time there is for** genetic changes or mutations to occur within their cells

Risk Factors

Cigarette Smoking and Tobacco Use

- Tobacco use is strongly linked to an increased risk for many kinds of cancer.
- Smoking cigarettes is the leading cause of the following types of cancer:
- Acute myelogenous leukemia (AML).
- Bladder cancer.

- Esophageal cancer.
- Kidney cancer.
- Lung cancer.
- Oral cavity cancer.
- Pancreatic cancer.
- Stomach cancer.
- Not smoking or quitting smoking lowers the risk of getting cancer and dying from cancer.
- Scientists believe that cigarette smoking causes about 30% of all cancer deaths in the United States.

Infections

- Certain viruses and bacteria are able to cause cancer. Viruses and other infection - causing agents cause more cases of cancer in the developing world (about 1 in 4 cases of cancer) than in developed nations (less than 1 in 10 cases of cancer).
- Examples of cancer-causing viruses and bacteria include:
- Human papillomavirus (HPV) increases the risk for cancers of the cervix, penis, vagina, anus, and oropharynx.
- Hepatitis B and hepatitis C viruses increase the risk for liver cancer.
- Epstein-Barr virus increases the risk for Burkitt lymphoma.
- Helicobacter pylori increases the risk for gastric cancer.
- Two vaccines to prevent infection by cancer-causing agents have already been developed and approved by the U.S. Food and Drug Administration (FDA).
- One is a vaccine to prevent infection with hepatitis B virus. The other protects against infection with strains of human papillomavirus (HPV) that cause cervical cancer.

Radiation

- Being exposed to radiation is a known cause of cancer. There are two main types of radiation linked with an increased risk for cancer:
- Ultraviolet radiation from sunlight: This is the main cause of nonmelanoma skin cancers.
- Ionizing radiation including: Medical radiation from tests to diagnose cancer such as x-rays, CT scans, fluoroscopy, and nuclear medicine scans.
- Radon gas in our homes.
- Scientists believe that ionizing radiation causes leukemia, thyroid cancer, and breast cancer in women. Ionizing radiation may also be linked to myeloma and cancers of the lung, stomach, colon, esophagus, bladder, and ovary. Being exposed to radiation from diagnostic x-rays increases the risk of cancer in patients and x-ray technicians.
- The growing use of CT scans over the last 20 years has increased exposure to ionizing radiation. The risk of cancer also increases with the number of CT scans a patient has and the radiation dose used each time.

The immune system

- People who have **weakened immune systems are more at risk of developing some types of cancer**.
- This includes people who **have had organ transplants and take drugs to suppress their immune systems to stop organ rejection**, plus people **who have HIV or AIDS, or other medical conditions** which reduce their immunity to Disease.

Diet

- The foods that you eat on a regular basis make up your diet. Diet is being studied as a risk factor for cancer. It is hard to study the effects of diet on cancer because a person's diet includes foods that may protect against cancer and foods that may increase the risk of cancer.
- It is also hard for people who take part in the studies to keep track of what they eat over a long period of time. This may explain why studies have different results about how diet affects the risk of cancer.
- Some studies show that fruits and nonstarchy vegetables may protect against cancers of the mouth, esophagus, and stomach. Fruits may also protect against lung cancer.
- Some studies have shown that a diet high in fat, proteins, calories, and red meat increases the risk of colorectal cancer, but other studies have not shown this.
- It is not known if a diet low in fat and high in fiber, fruits, and vegetables lowers the risk of colorectal cancer.

Alcohol

- Studies have shown that drinking alcohol is linked to an increased risk of the following types of cancers:
  Oral cancer.
  Esophageal cancer.
  Breast cancer.
  Colorectal cancer (in men).
  Drinking alcohol may also increase the risk of liver cancer and female colorectal cancer.

Physical Activity

- Studies show that people who are physically active have a lower risk of certain cancers than those who are not. It is not known if physical activity itself is the reason for this.
- Studies show a strong link between physical activity and a lower risk of colorectal cancer. Some studies show that physical activity protects against postmenopausal breast cancer and endometrial cancer.

Obesity

Studies show that obesity is linked to a higher risk of the following types of cancer:

- Postmenopausal breast cancer.
- Colorectal cancer.
- Endometrial cancer.

- Esophageal cancer.
- Kidney cancer.
- Pancreatic cancer.
- Some studies show that obesity is also a risk factor for cancer of the gallbladder.
- It is not known if losing weight lowers the risk of cancers that have been linked to obesity.

Environmental Risk Factors

- Being exposed to chemicals and other substances in the environment has been linked to some cancers:
- Links between air pollution and cancer risk have been found. These include links between lung cancer and secondhand tobacco smoke, outdoor air pollution, and asbestos.
- Drinking water that contains a large amount of arsenic has been linked to skin, bladder, and lung cancers.
- Studies have been done to see if pesticides and other pollutants increase the risk of cancer. The results of those studies have been unclear because other factors can change the results of the studies.

Chemicals/Environment

- Asbestos Exposure and Cancer Risk
- Agricultural Health Study
- Formaldehyde and Cancer Risk
- Hair Dyes and Cancer Risk
- Cancer Clusters
- Farmers, farm workers, and farm family members may be exposed to substances such as pesticides, engine exhausts, solvents, dusts, animal viruses, fertilizers, fuels, and specific microbes that may account for these elevated cancer rates.Farming communities have higher rates of leukemia, non-Hodgkin lymphoma, multiple myeloma, and soft tissue sarcoma, as well as cancers of the skin, lip, stomach, brain, and prostate.

- Formaldehyde is a colorless, flammable, strong-smelling chemical that is used in building materials and to produce many household products. It is used in pressed-wood products, such as particleboard, plywood, and fiberboard; glues and adhesives; permanent-press fabrics; paper product coatings; and certain insulation materials.

- In addition, formaldehyde is commonly used as an industrial fungicide, germicide, and disinfectant, and as a **preservative in mortuaries and medical laboratories.**

- Formaldehyde also occurs naturally in the environment. It is produced in small amounts by most living organisms as part of normal metabolic processes.

- Asbestos has been classified as a known human carcinogen (a substance that causes cancer) by the U.S. Department of Health and Human Services, the EPA, and the International Agency for Research on Cancer.
- Studies have shown that exposure to asbestos may increase the risk of lung cancer and mesothelioma (a relatively rare cancer of the thin membranes that line the chest and abdomen).
- Although rare, mesothelioma is the most common form of cancer associated with asbestos exposure. **In addition to lung cancer and mesothelioma**, some studies have suggested an association between asbestos exposure and gastrointestinal and colorectal cancers, as well as an elevated risk for cancers of the throat, kidney, esophagus, and gallbladder.
- However, the evidence is inconclusive.
- Other substances in this category, including both naturally occurring and synthetic asbestos-like fibers, such as wollastonite, attapulgite, glass wool, and rock wool, are believed to have similar effects.
- Non-fibrous particulate materials that cause cancer include powdered metallic cobalt and nickel, and crystalline silica (quartz, cristobalite, and tridymite).
- Usually, physical carcinogens must get inside the body (such as through inhaling tiny pieces) and require years of exposure to develop cancer.

Hormones

- Insulin-like growth factors and their binding proteins play a key role in cancer cell proliferation, differentiation and apoptosis, suggesting possible involvement in carcinogenesis.
- Hormones are important agents in sex-related cancers, such as cancer of the breast, endometrium, prostate, ovary, and testis, and also of thyroid cancer and bone cancer.
- For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may explain why these women have higher risk of breast cancer, even in the absence of a breast-cancer gene.
- Similarly, men of **African ancestry have significantly higher levels of testosterone than men of European ancestry**, and have a correspondingly much **higher level of prostate cancer.** Men of Asian ancestry, with the **lowest levels of testosterone-activating androstanediol glucuronide, have the lowest levels of prostate cancer**.

- Other factors are also relevant: **obese people have higher levels of some hormones associated with cancer and a higher rate of those cancers**.
- Women who take hormone replacement therapy have a higher risk of developing cancers associated with those hormones.
- On the other hand, people who exercise far more than average have lower levels of these hormones, and lower risk of cancer.
- Osteosarcoma may be promoted by growth hormones.
- Some treatments and prevention approaches leverage this cause by artificially reducing hormone levels, and thus discouraging hormone-sensitive cancers

Heredity

- The vast majority of cancers are non-hereditary ("sporadic cancers").
- Hereditary cancers are primarily caused by an inherited genetic defect.
- Less than 0.3% of the population are carriers of a genetic mutation that has a large effect on cancer risk and these cause less than 3–10% of all cancer.

THE GENETICS OF CANCER

There are two basic types of genetic mutations:

- **Acquired mutations** are the most common cause of cancer. These occur from damage to genes during a person's life. They are not passed from parent to child. Factors such as tobacco, ultraviolet (UV) radiation, viruses, and age cause these mutations. Cancer that occurs because of **acquired mutations is called sporadic cancer.**
- **Germline mutations**, which **are less common**, are **passed directly from a parent to a child**. In these situations, the mutation **can be found in every cell of a person's body**, including the reproductive sperm cells in a boy's body and egg cells in a girl's body. Because the mutation affects reproductive cells, it passes from generation to generation. Cancer caused by **germline mutations is called inherited cancer**, and it makes up **about 5% to 10% of all cancers**.
- **Types of genes linked to cancer**
- Many of the genes that contribute to the development of cancer fall into broad categories:
- **Tumor suppressor genes** are protective genes. **Normally, they limit cell growth by monitoring how quickly cells divide into new cells, repairing mismatched DNA,**

and controlling when a cell dies (a process known as *apoptosis* or *programmed cell death)*.

- When a tumor suppressor gene is mutated, cells grow uncontrollably and may eventually form a mass called a tumor.
- *BRCA1*, *BRCA2*, and *p53* are examples of tumor suppressor genes.
- Germline mutations in *BRCA1* or *BRCA2* genes increase a woman's risk of developing hereditary breast or ovarian cancers.
- The most commonly mutated gene in people who have cancer is *p53*.
- In fact, more than 50% of all cancers involve a missing or damaged *TP53* gene – CODE P53 PROTEIN. Most T*p53* gene mutations are acquired mutations. Germline T*p53* mutations are rare.

- ❑ It may be helpful to think of a cell as a car. For it to work properly, there need to be ways to control how fast it goes. **A proto-oncogene normally functions in a way that is much like a gas/fuel pedal.** It helps the cell grow and divide. **An oncogene could be compared with a gas/fuel peda**l that is stuck down, which causes the cell to divide out of control.
- ❑ A tumor suppressor gene is like the brake pedal on a car. It normally keeps the cell from dividing too quickly, just as a brake keeps a car from going too fast. When something goes wrong with the gene, such as a mutation, cell division can get out of control.
- ❑ An important difference between oncogenes and tumor suppressor genes is that **oncogenes result from the *activation* (turning on)** of proto-oncogenes, but tumor suppressor genes cause cancer when they are ***inactivated* (turned off).**

Oncogenes

- **Proto-oncogenes are genes that normally help cells grow**.
- When a **proto-oncogene mutates (changes**) or there are too many copies of it, it **becomes a "bad" gene** that can become permanently turned on or activated when it is not supposed to be. When this happens, the cell grows out of control, which can lead to cancer. This bad gene is called an oncogene.
- A few cancer syndromes are caused by inherited mutations of proto-oncogenes that cause the oncogene to be turned on (activated). But most cancer-causing mutations involving **oncogenes are acquired**, not inherited.

They generally activate oncogenes by:

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Oncogenes turn a healthy cell into a cancerous cell. **Mutations in these genes are not inherited**.

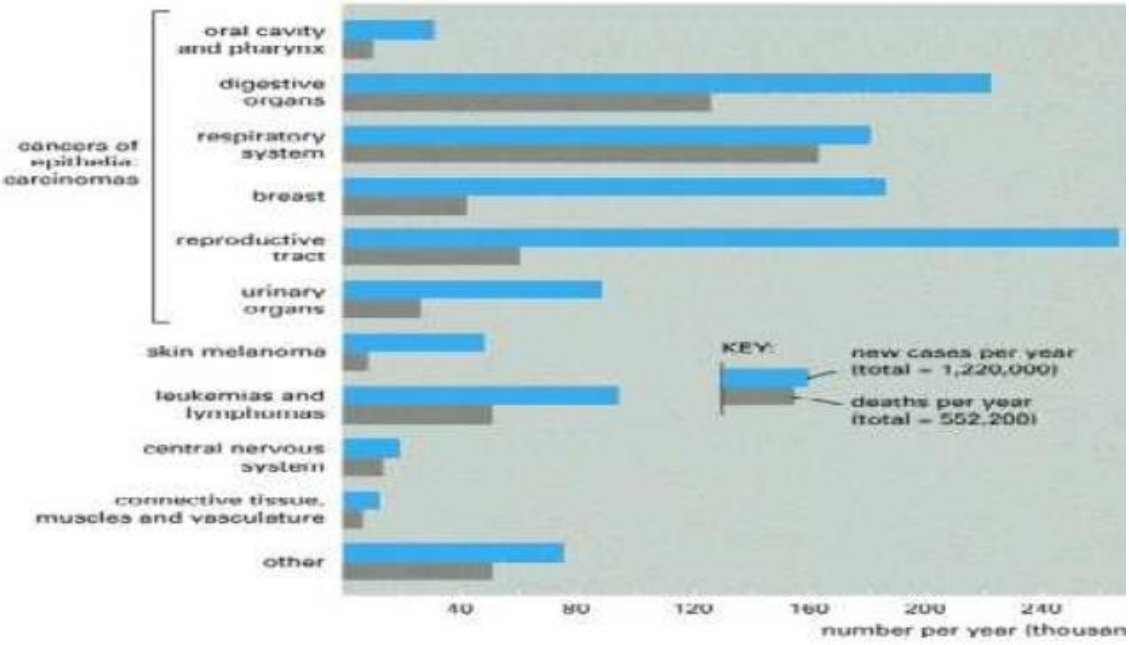
Two common oncogenes are:

- *HER2*, which is a specialized protein that controls cancer growth and spread, and it is found on some cancer cells, such as breast and ovarian cancer cells
- *T*he ***ras*** **family of genes**, which make proteins involved in cell communication pathways, cell growth, and cell death.
- DNA repair genes fix mistakes made when DNA is copied. But if a person has an error in a DNA repair gene, these mistakes are not corrected. And then they become mutations, which may eventually lead to cancer. **This is especially true if the mutation occurs in a tumor suppressor gene or oncogene**.
- Mutations in DNA repair genes can be inherited, such as with Lynch syndrome, or acquired.

Despite all that is known about the different ways cancer genes work, many cancers cannot be linked to a specific gene. Cancer likely involves multiple gene mutations. Some evidence also suggests that genes interact with their environment, further complicating genes' role in cancer.

Telomere Shortening May Pave the Way to Cancer in Humans

The great majority of mouse cancers are sarcomas and leukemias, whereas more than 80 percent of human cancers are carcinomas—cancers of epithelia where rapid cell turnover occurs.



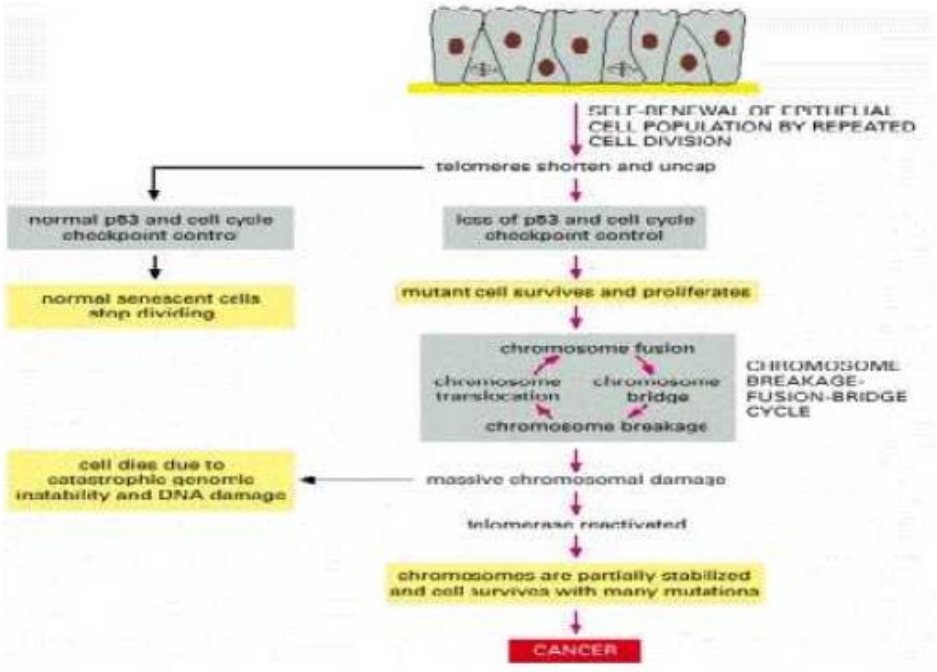
Many therapies have been found to cure cancers in mice; but when the same treatments are tried in humans, they usually fail. What could be the reason for the difference between mouse and human cancer, and what can it tell us about the molecular mechanisms of the disease?

An important part of the answer may lie in the

1. Behavior of telomeres and the relationship between telomere shortening,
2. Replicative cell senescence,
3. Genetic instability.

- Human cells seem to have a built-in limit to their proliferation: they show **replicative senescence**, at least when grown in culture.
- Replicative cell senescence in humans is thought to be caused by changes in the structure of telomeres—the repetitive DNA sequences and associated proteins that cap the ends of each chromosome.

- These telomeric DNA sequences are synthesized and maintained by a special mechanism that requires the enzyme telomerase
- In most human cells, **other than those of the germ line and some stem cells, expression of the gene coding for the catalytic subunit of telomerase is switched off**, or **at least not fully activated**.
- As a result, **the telomeres in these cells tend to become a little shorter** with each round of cell division.
- Eventually, **the telomeric cap on the chromosome end can become shortened to the point where a danger signal is generated, arresting the cell cycle**.
- **The signal is similar, in function at least, to the one that arrests** the cycle **when an uncapped DNA end is created** by an accidental double-strand chromosome break.
- The **effect in both cases is to prevent cell division** so long as the **cell contains broken or inadequately capped DNA.**
- In the cell with the chromosome break, this allows time for DNA repair; in the normal senescent cell, it seems that it simply puts a stop to cell proliferation.



- it is not clear how often cells in normal human tissues run up against this limit; but if a self-renewing cell population does undergo replicative senescence, any rogue cell that undergoes a mutation that lets it carry on dividing will enjoy a huge competitive advantage—much more than if the same mutation had occurred in a cell in a nonsenescent population.

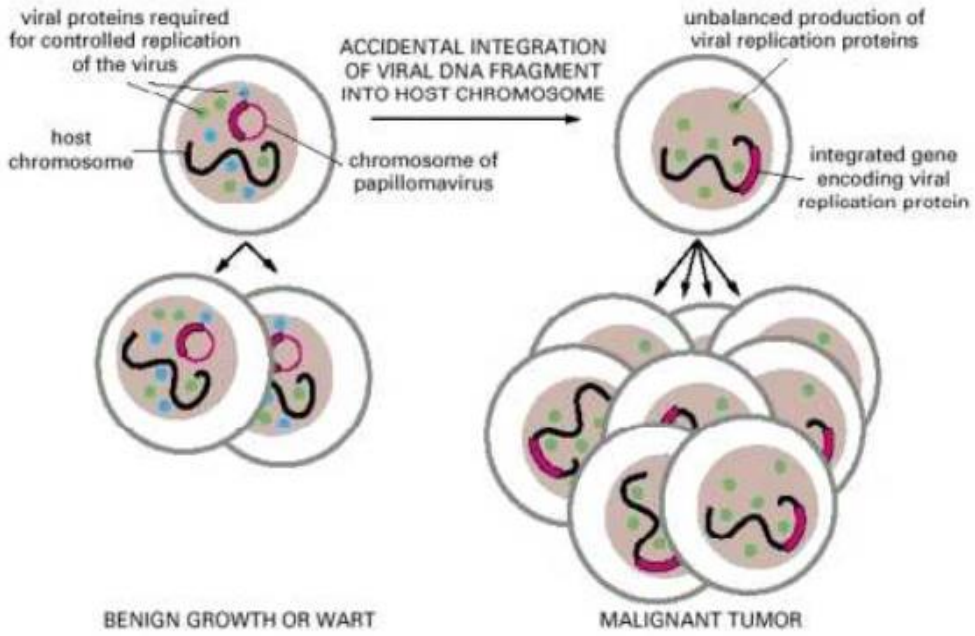
- Viewed in this light, replicative senescence might be expected to favor the development of cancer.
- Mice have telomeres much longer than those of humans. Moreover, unlike humans, they **keep telomerase active in their somatic cells, and mouse telomeres therefore do not tend to shorten with increasing age of the organism.**
- It is possible, however, to use gene knockout technology to make mice that lack functional telomerase.
- In these mice, **the telomeres become shorter with every generation, but no untoward consequences are seen until**, in the great-great-grandchildren of the initial mutants, the telomeres become so short that they disappear or cease to function.
- Beyond this point**, the mice begin to show various abnormalities, including an increased incidence of cancer.**
- This raises the possibility that natural telomere shortening helps to engender many human tumors

DNA Tumor Viruses

- **DNA tumor viruses** cause cancer mainly by interfering with cell-cycle controls, including those that depend on p53.
- To understand this type of viral carcinogenesis, it is important to understand the life history of the virus.
- **Viruses use the DNA replication machinery of the host cell to replicate their own genomes**.
- To make many infectious virus particles from a single host cell, a DNA virus has to commandeer this machinery and drive it hard, **breaking through the normal constraints on DNA replication** and usually **killing the host cell in the process**.
- **Typically, however, the virus also has another option: it can propagate its genome as a quiet,** well-behaved passenger in the host cell, replicating in parallel with the host cell's DNA in the course of ordinary cell division cycles.
- The virus can switch between **these two modes of existence**, **remaining latent** and **harmless** or **proliferating to generate infectious particles according to circumstances. No matter which way of life the virus is following, it is not in its interests to cause cancer**. But **genetic accidents can occur**, such that the **virus misuses its equipment for commandeering the DNA replication machinery**, and **instead of switching on rapid replication of its own genome, switches on persistent proliferation of the host cell**.

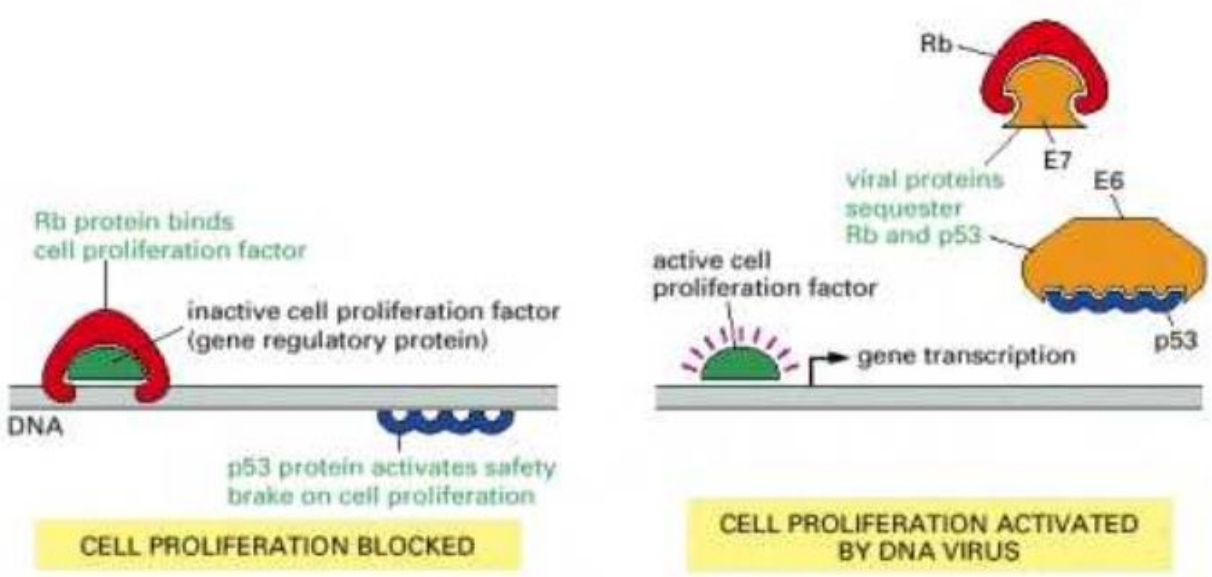
DNA tumor viruses- The *papillomaviruses*

- **The *papillomaviruses,*** for example, are the cause of human warts and are especially important as a key causative factor in carcinomas of the uterine cervix (about 6% of all human cancers).
- Papillomaviruses **infect the epithelium, and are retained in the basal layer of cells as extrachromosomal plasmids that replicate in step with the chromosomes**. Infectious virus particles are generated in the outer epithelial layers, as cells begin to differentiate before being sloughed from the surface.
- Here, cell division should normally be arrested, but the virus interferes with this arrest so as to allow rapid replication of its own genome.
- Usually, the effect is restricted to the outer layers of cells and relatively harmless, as in a wart.
- Occasionally, through a **genetic accident causing misregulation** of the viral genes whose **products prevent cell-cycle arrest**, **the control of cell division** is **subverted in the basal layer also, in the stem cells of the epithelium**.
- This can lead to cancer, with the viral genes acting as oncogenes



Papillomaviruses have double-stranded circular DNA chromosomes of about 8000 nucleotide pairs. In a wart or other benign infection these chromosomes are stably maintained in the basal cells of the epithelium as plasmids whose replication is regulated so as to keep step with the chromosomes of the host (left). Rare accidents can cause the integration of a fragment of such a plasmid into a chromosome of the host, altering the environment of the viral genes. This (or possibly some other cause) disrupts the control of viral gene expression. The unregulated production of viral replication proteins interferes with the control of cell division, thereby helping to generate a cancer (right).

- In papillomaviruses, the viral genes that are mainly to blame are called ***E6* and *E7***.
- The products of these viral oncogenes interact with many host cell proteins, **but in particular they bind to the protein products of two key tumor suppressor genes of the host cell**, putting them out of action and so permitting the cell to replicate its DNA and divide in an uncontrolled way.
- One of these **host proteins is Rb: by binding to Rb, the viral E7 protein prevents** it from binding to its normal associates in the cell. The other **host protein inactivated by the virus is the tumor suppressor p53**, which is **bound by the viral E6 protein, triggering p53 destruction**.
- Elimination of **p53 allows the abnormal cell to survive, divide, and accumulate yet more abnormalities**.



SIGNS AND SYMPTOMS OF CANCER

As there are so many different types of cancer the symptoms are varied and depend on where the disease is located. However, there are some key signs and symptoms, including:

•**Lumps**–some cancers can be felt through the skin.Cancerous lumps are often painless and may increase in size as the cancer progresses

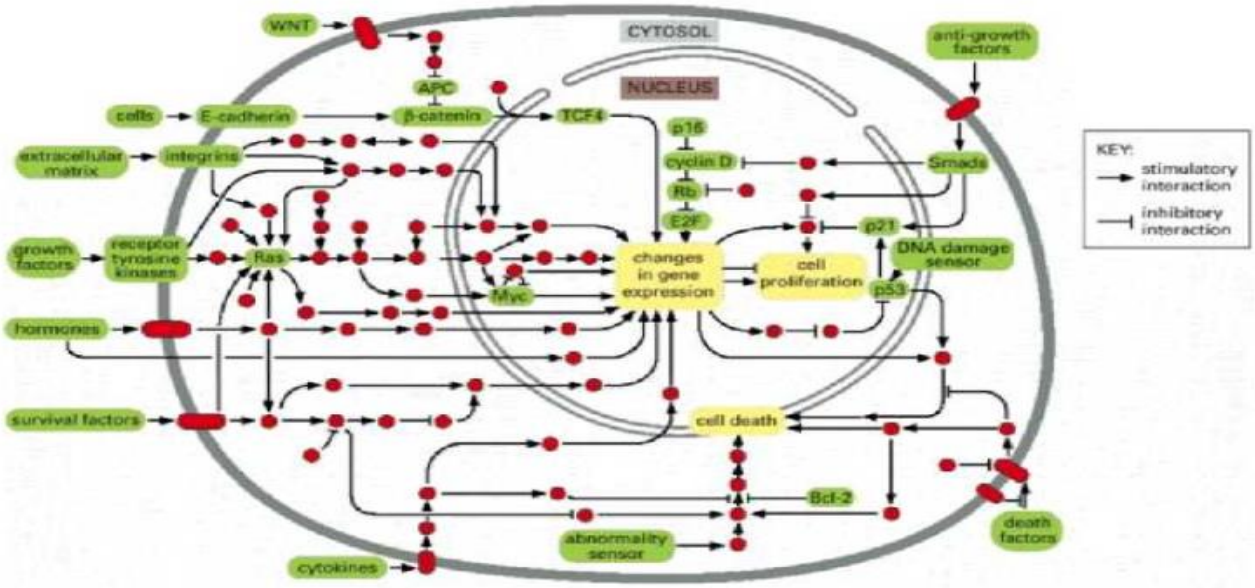
•**Coughing, breathlessness** –persistent coughing episodes and breathlessness can be associated with lung cancer.

•**Changes in bowel habit–symptoms** of bowel cancer may include blood in the stools and a change in bowel habits such as constipation and diarrhoea.

CLASSIFICATION OF CANCER GENES

Many Cancer-Critical Genes Regulate Cell Division

- Most cancer-critical genes code for **components of the pathways** that regulate the **behavior of cells** in the body—in particular, the **mechanisms by which signals from a cell's neighbors can impel it to divide, differentiate, or die** .
- In fact, many of the components of **cell-signaling pathways were first identified through searches for cancer-causing genes,** and a full list of **proto-oncogene products and tumor suppressors includes.**
- Examples : molecule involved in cell **signaling—secreted proteins, transmembrane receptors, GTP-binding proteins, protein kinases, gene regulatory proteins, and so on .**
- Many **cancer mutations alter signal pathway components** in a way **that causes them to deliver proliferative signals even when more cells are not needed,** switching on cell growth, DNA replication, and cell division inappropriately.
- Mutations that inappropriately **activate a receptor tyrosine kinase,** such as the **EGF** receptor, or proteins in the **Ras (sig ptn) family**, which **lie downstream from such growth factor receptors,** act in this way.



Products of both oncogenes and tumor suppressor genes often occur within the same pathways. Individual signaling proteins are indicated by *red circles*, with the cancer-critical components and control mechanisms discussed in this chapter in *green*. Stimulatory and inhibitory interactions between proteins are designated as shown in the key.

Cell Cycle - General Overview

A eukaryotic cell cannot divide unless two important processes alternate:

Doubling of its *genome* (DNA) in *synthesis* phase (**S Phase**) of the cell cycle

Halving of the *genome* during *mitosis* (**M Phase**).

The phase between M and S is called $\mathbf{G_1}$, and the phase between S and M is $\mathbf{G_2}$

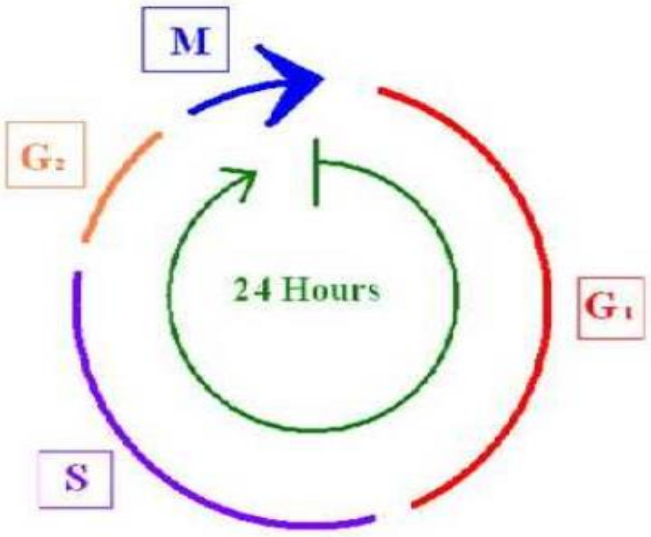
And so, the 4 phases of the cell cycle are:

$\mathbf{G_1}$ => growth and preparation of the chromosomes for replication

S => synthesis of DNA and duplication of the centrosome

$\mathbf{G_2}$ => preparation for mitosis

M => division into two identical daughter nuclei

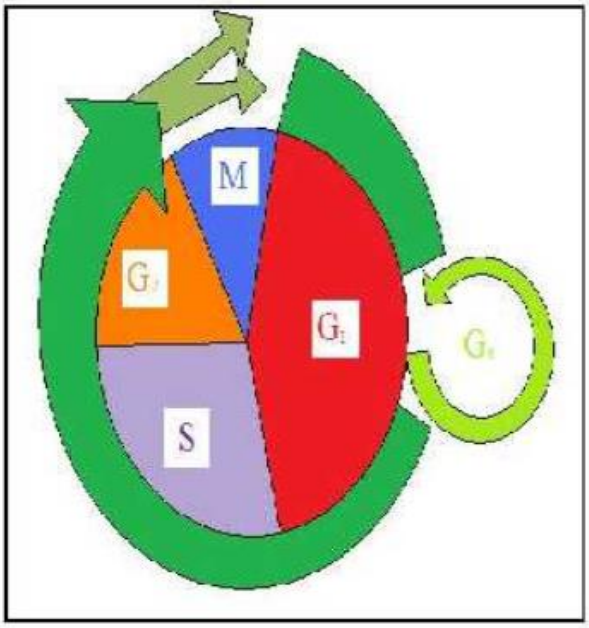


The **passage of a cell through the cell cycle is controlled by proteins in the cytoplasm. The main proteins involved in animal cells are:**

Cyclins

G_1 cyclins (cyclin D)
S phase cyclins (cyclins E and A)
Mitotic cyclins (cyclin B)
Cyclin-dependent kinases (CDKs)
a G_1 CDK (CDK4/6)
an S phase CDK (CDK2)
and M phase CDK (CDK1)
Cell cyclin inhibitors
cip/kip family e.g., p21, p27, p51
INK4a/ARF family e.g., p16INK4a

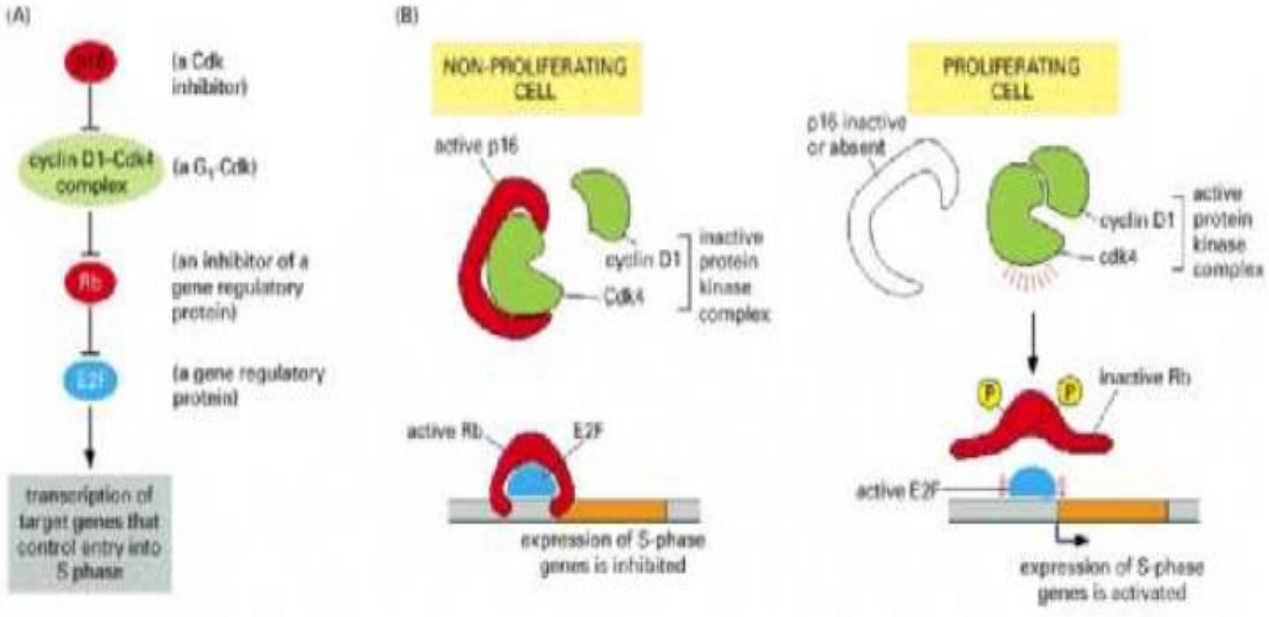
Other proteins involved in the cell cycle are:
Retinoblastoma protein
The anaphase-promoting complex (APC)



- The **cancer-critical genes** that **regulate** cell **division** exert their effects by acting on the central cell-cycle **control machinery**.
- Mutations in this machinery feature prominently in many cancers.
- A key point at which cells make the decision to replicate their DNA and enter the cell division cycle is thought to be **controlled by the Rb protein**, the product of the tumor suppressor gene *Rb*.
- **Rb serves as a brake that restricts** entry into S **phase** by binding to **gene regulatory proteins needed to express genes whose products are required for progress round the cycle.**

- Normally, this **inhibition by Rb is relieved** at the appropriate time by **phosphorylation of Rb**, which causes it **to release its inhibitory grip**.
- Many **cancer cells proliferate inappropriately by eliminating Rb entirely**.
- **Other tumors achieve the same endpoint by acquiring mutations in other components of the Rb regulatory pathway**.
- Thus, in normal cells, **a complex of cyclin D1** and the **cyclin-dependent kinase Cdk4 (G_1-Cdk) stimulates** progression through the cell cycle by **phosphorylating Rb.**

- The **p16 (INK4) protein—which** is produced **when cells are stressed—inhibits cell-cycle progression by preventing the formation of an active cyclin D1-Cdk4 complex.**
- Some **glioblastomas and breast cancers are found to have amplified the genes encoding Cdk4 or cyclin D1, thus favoring cell proliferation.**
- **And deletion or inactivation of the *p16* gene is common in many forms of human cancer**.
- In cancers **where it is not inactivated by mutation, this gene is often silenced by methylation of its regulatory DNA**.



All the components of this pathway have been found to be altered by mutation in human cancers (**products of proto-oncogenes, *green;* products of tumor suppressor genes, *red;* E2F shown in *blue* because it has both inhibitory and stimulatory actions, depending on the other proteins that are bound to it**). In most cases, only one of the components is altered in any individual tumor. (A) A simplified view of the dependency relationships in this pathway

(B) The Rb protein inhibits entry into the cell-division cycle when it is **unphosphorylated.The complex of Cdk4 and cyclin D1 phosphorylates Rb, thereby encouraging cell proliferation**. When a cell is stressed, p16 inhibits the formation of an active Cdk4/cyclin D1 complex, preventing proliferation. **Inactivation of Rb or p16 by mutation encourages cell division (thus each can be regarded as a tumor suppressor) while overactivity of Cdk4 or cyclin D1 encourages cell division** (thus each can be regarded as a proto-oncogene).

Signaling pathways can function to inhibit cell division,

- The best known example being the **antigrowth effect of the TGFB (***Transforming growth factor beta* –MULTIFUNCTIONAL CYTOKINE)**family of signaling proteins**
- Loss of growth inhibition through TGFβ-mediated pathways contributes to the genesis of several types of human cancers.
- The receptor **TGFβ-RII is found to be mutated in some cancers of the colon**
- **Smad4—a key intracellular signal transducer in the pathway—is** inactivated in cancers of the **pancreas and some other tissues.**
- The variety of ways in which the machinery of cell-cycle control can be altered in **cancer illustrates two important points**.
- First, **it explains why individual cases of a particular cancer showing the same symptoms may arise from different mutations**: in **many cases several alternative mutations will have much the same effect on cell proliferation.**
- Second, **it reinforces the point that there is no fundamental difference in the processes that are affected by oncogenes—which become activated by mutation—and those affected by tumor suppressor genes**—which become inactivated.

- These **two classes of cancer-critical genes merely differ in whether they play a stimulatory or inhibitory role in a pathway**

P53 and Cancer

- The control of the cell cycle is achieved by many proteins present in the cytoplasm.
- **p53**, or protein 53, is a multifunctional tumour suppressor protein encoded by the TP53 gene in humans, mapped to the short arm of chromosome 17.

- It is often described as *'the guardian of the genome'* , as it has an **important role in conserving the genome by preventing mutation**.
- The name **p53 comes from the molecular mass of the protein, which was measured to be 53K Dalton** when it was initially discovered

History

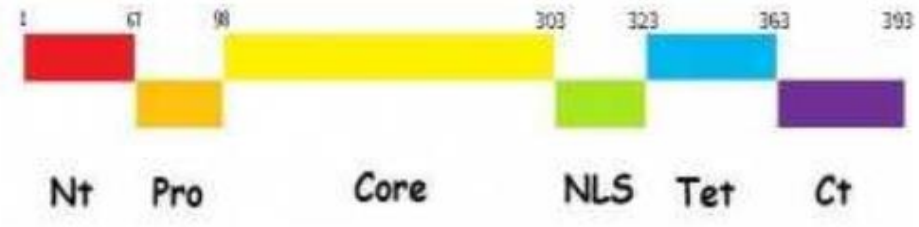
- *1979*: **p53 first discovered** independently by David Lane and Arnold Levine as a **cellular protein in complex with the T-antigen of SV40**
- *1983-1984*: **p53 was isolated and cloned**, and was found to cooperate with other **oncogene *in vitro* transformation assays.**
- *1980s*: Several observations that **p53 was a oncogene**.
- *1984*: Warren Maltzman first **recognised that DNA damage in the form of ultraviolet (UV) radiation increased levels of p53 in cells**.
- *1989*: Bert Vogelstein and colleagues **reported there was frequent 'loss-of-heterozygosity' at the p53 locus (TP53) in a series of human colorectal cancers and suggested p53 was not an oncogene, but a tumour suppressor gene (TSG)**.
- *Early 1990s*: **More confirming observations that TP53 is a TSG from analysis of many tumours of many different types:-** in 1990, inherited cancer pre-disposing syndrome in humans, Li-Fraumeni (LFS) was due to germline mutation in TP53; in 1992, Mice genetically deficient in TRP53 (the mouse p53 gene) were extremely tumour prone.

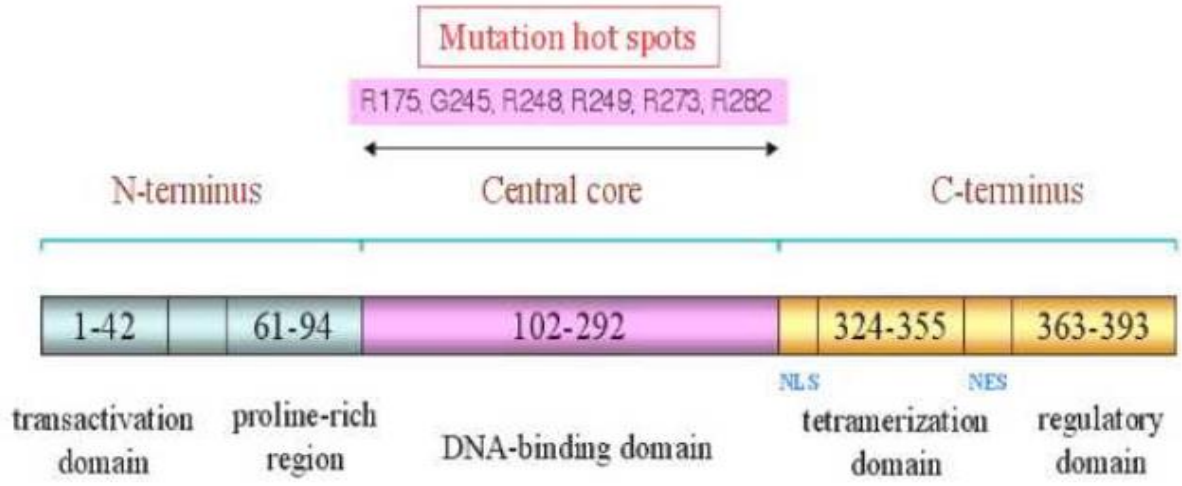
Structure of p53

The p53 protein is a polypeptide of approximately 400 amino acids, and is 393 amino acids long for a human p53.

It consists of several domains:

- N-terminal transcription activation domain (TAD), which can be further divided into two smaller sub-domains, TAD-I (residues 1-40) and TAD-II (residues 41-67)
- Proline-rich region (residues 67-98)
- Central core domain (residues 98-303)
- Nuclear localisation signal containing region (residues 303-323)
- Oligomerisation domain (residues 323-363) and
- C-terminal basic domain (residues 363-393).





AMINO TERMINAL DOMAIN

- transactivation
- transcription factors – MDM2

PROLINE-RICH REGION

- stability

CENTRAL CORE

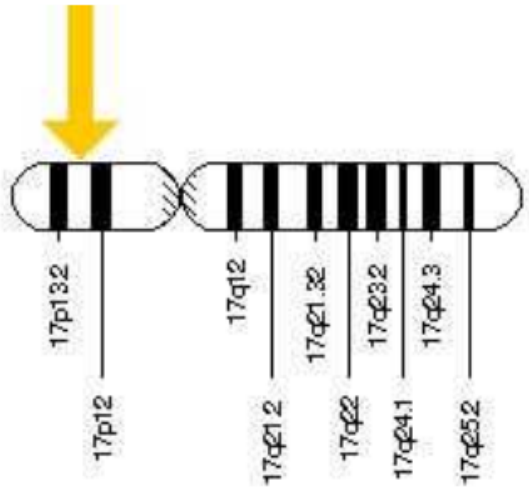
- DNA binding domain

C-TERMINAL DOMAIN

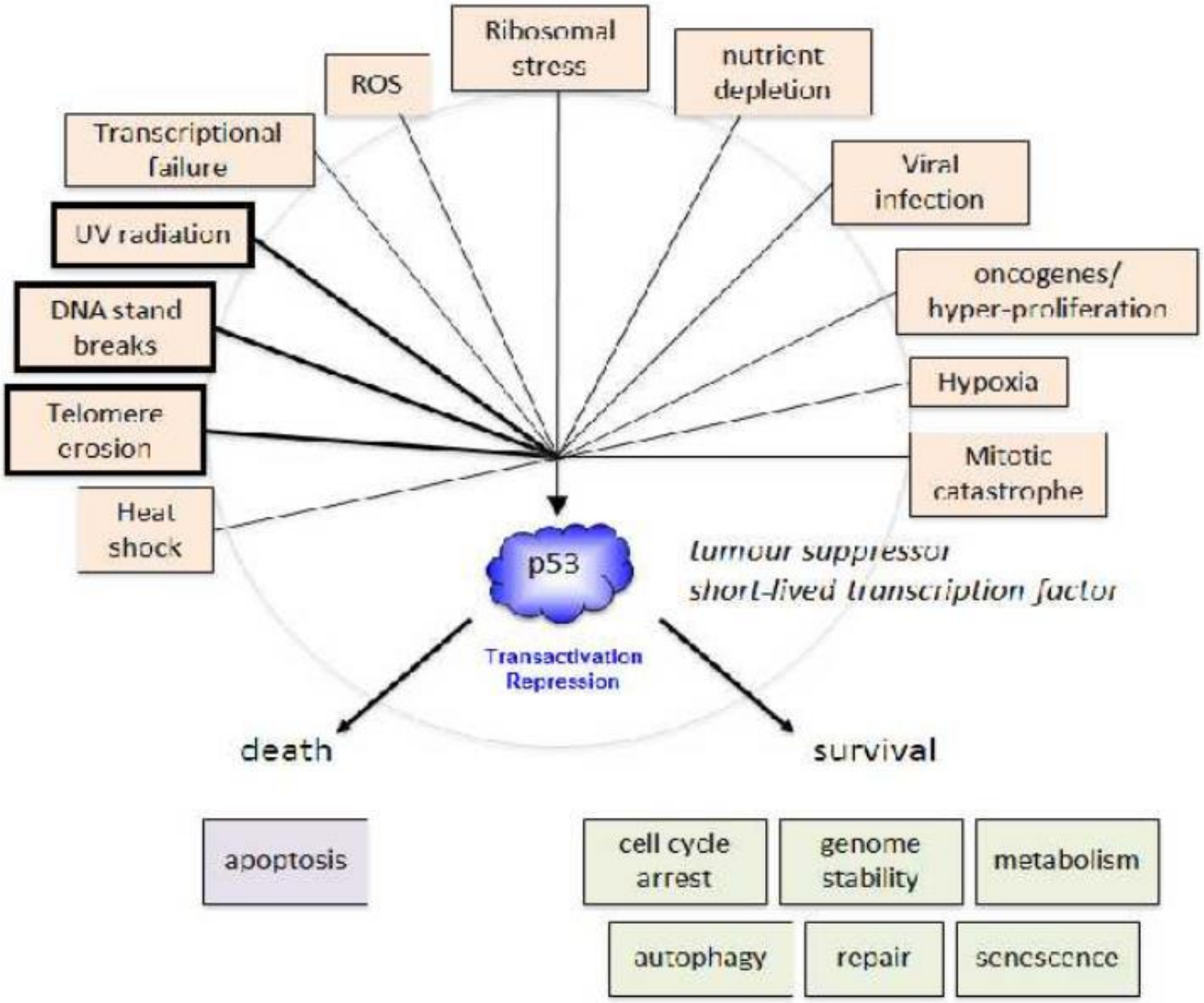
- negative regulation

Where is the TP53 gene located?

- Molecular Location on chromosome 17: base pairs 7,668,401 to 7,687,549
- The TP53 gene is located on the short (p) arm of chromosome 17 at position 13.1.
- More precisely, the TP53 gene is located from base pair 7,668,401 to base pair 7,687,549 on chromosome 17.

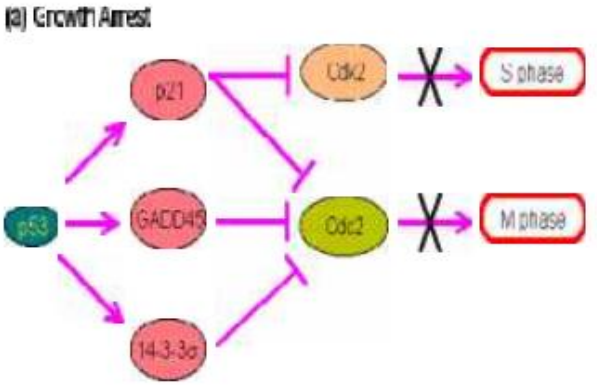


Function of p53



1. Its main function is to **regulate the cell cycle by preventing a cell from completing the cycle when the DNA is damaged**. This **'growth arrest'** function of p53 is **important in preventing cancer by suppressing tumours,** and **p53 mutations or non-functioning p53 are observable in many human cancers.**
2. If the **damage can be repaired, p53 starts a cascade of events that induces cell cycle arrest providing more time for DNA repair**.
3. If **the damage cannot be repaired, it triggers cell to undergo apoptosis (cell death).**

- p53 regulates the cell cycle by binding to the DNA to produce a protein called **p21, also known as cyclin-dependent kinase inhibitor** 1A (CDKN1A).
- This protein **can interact with cyclin-dependent kinases (CDKs), CDK2 and CDK4 in particular**.
- When this is complexed with CDKs, it **functions as an inhibitor to interfere with the activities of CDKs to delay the progression of each phases.**
- By doing this, **p53 can prevent the replication of cells to give the cell more time to repair**. It also **activates the transcription of some of the proteins involved in *DNA repair***, such as **ribonucleotide reductase which is encoded by the p53R2 gene.**
- If this **repair effort fails, more p53 accumulates and this increase in p53 protein will guide the cell to cell apoptosis**.



a) The cell cycle progression into the S phase requires the **enzyme Cdk2,** which can **be inhibited by p21**.

The progression into the M phase requires Cdc2 which can be inhibited by p21, GADD45 or 14-3-3s. p53 regulates the expression of these inhibitory proteins to induce growth arrest.

(b) Apoptosis can be induced by the binding of Caspase 9 to cytochrome c and Apaf1. p53 may activate the expression of Apaf1 and Bax. **The latter can then stimulate the release of cytochrome c from mitochondria.**

Target Genes of p53

p53 is a transcriptional activator, regulating the expression of **Mdm2 (**Mouse double minute 2 homolog) for its own regulation and the genes involved in growth arrest, DNA repair and apoptosis.

Some important examples are listed below.

1. **Growth arrest:** p21, Gadd45(The Growth Arrest and DNA Damage), and 14-3-3s(14-3-3 proteins eluted in the 14th fraction of bovine brain homogenate and were found on positions 3.3 of subsequent electrophoresis).
2. **DNA repair:** p53R2.
3. **Apoptosis:** Bax, Apaf-1(Apoptotic protease activating factor 1), PUMA(p53 upregulated modulator of apoptosis)) and NoxA.

Abnormalities related to p53:

- p53 tightly controls the expression of the gene which encodes for p21 in response to a variety of stimuli.
- If **p53 is damaged or mutated, it can no longer bind to the DNA to operate as the 'stop signal' for cell division**. **This results in the formation of tumours as cells divide uncontrollably.**
- **Genetic alterations found in the central core domain of p53**, which results in the loss of sequence-specific DNA binding activity, are the most commonly observed mutations in human cancer.

Current and Future Research

- Since this paradigm shift, a lot of study has been focused on the investigation of the p53 signalling pathways, mechanisms of action and how p53 functions as a tumour suppressor protein.
- It was also voted *molecule of the year* by Science magazine.
- A diverse range of information has been collected to present and currently, there are more than 49,000 papers on p53available.
- However, the complete understanding of how p53 functions is not yet fully known, and only general patterns have emerged over the years.
- There are 'International p53 Workshops' held every two years, in different cities around the world.
- These workshops provide a forum for researchers to discuss and share their latest findings relevant to p53, and provide new information for students and general public.

- At present, p53 has been recognised as a key tumor suppressor and important target for novel cancer therapy.
- Future research will continue to focus on the role of p53 in cancer therapeutics, identifying drug targets applicable to p53, and influencing the p53 activation cascade.
- In the near future, we may see the use of p53 in the diagnosis and treatment of cancer.

How are changes in the TP53 gene related to health conditions?

Breast cancer - increased risk from variations of the TP53 gene

Inherited changes in the TP53 gene greatly increase the **risk of developing breast cancer as part of a rare cancer syndrome called Li-Fraumeni syndrome(**his syndrome is also known as the **sarcoma, breast, leukaemia and adrenal gland (SBLA) syndrome)** .

- These inherited mutations are thought to account for less than 1 percent of all breast cancer cases.
- **Non-inherited (somatic) mutations in the TP53 gene are much more common**, occurring in approximately 20 to 40 percent of all breast cancer cases.
- These **somatic mutations are acquired during a person's lifetime and are present only in tumor cells**. The cancers associated with somatic mutations are typically not inherited and do not occur as part of a cancer syndrome.
- Li-Fraumeni syndrome - associated with the TP53 gene
- Although somatic mutations in the TP53 gene are found in many types of cancer, Li-Fraumeni syndrome appears to be the only cancer syndrome associated with inherited mutations in this gene.
- More than **60 different mutations in the TP53 gene have been identified in individuals with Li-Fraumeni syndrome**. These mutations are typically inherited from a parent and are present in all of the body's cells.
- Many of the mutations associated with Li-Fraumeni syndrome change single amino acids in the part of the p53 protein that binds to DNA.
- Other mutations delete small amounts of DNA from the gene.
- Mutations in the TP53 gene lead to a version of p53 that cannot effectively regulate cell growth and division. Specifically, the altered protein is unable to trigger apoptosis in cells with mutated or damaged DNA. As a result, DNA damage can accumulate in cells. Such cells may continue to divide in an uncontrolled way, leading to the growth of tumors.

- Many of these mutations change single protein building blocks (amino acids) in the p53 protein.
- These mutations **lead to the production of a nonfunctional version of this protein**. The defective protein builds up in cells and cannot regulate cell growth and division.
- In some cases of breast cancer, **one copy of the TP53 gene is lost and the remaining copy has a mutation that prevents the cell from producing any p53.**
- Without this protein, **DNA damage accumulates and cells divide in an uncontrolled way, leading to a cancerous tumor.**
- Mutations in the **TP53 gene are associated with larger tumors and more advanced disease than breast cancers without TP53 mutations.**
- Recurring tumors are also more likely to have mutations in the TP53 gene.

Bladder cancer - associated with the TP53 gene

- Somatic TP53 gene mutations in bladder cells have been found in some cases of bladder cancer.
- Most of these mutations change single amino acids in p53.
- The altered protein cannot bind to DNA, preventing it from effectively regulating cell growth and division.
- As a result, DNA damage accumulates in cells. These abnormal cells may divide in an uncontrolled way, leading to the growth of a cancerous tumor. Mutations in the TP53 gene may help predict whether bladder cancer will progress and spread to nearby tissues, and whether the disease will recur after treatment.
- head and neck squamous cell carcinoma - associated with the TP53 gene
- ***Somatic mutations in the TP53 gene have been found in nearly half of all head and neck squamous cell carcinomas*** (HNSCC).
- This type of cancerous tumor occurs in the moist lining of the mouth, nose, and throat.
- Most of the TP53 gene mutations involved in HNSCC change single amino acids in p53; these changes impair the protein's function. Without functioning p53, DNA damage builds up in cells, and they can continue to divide without control, leading to tumor formation.

- other cancers - associated with the TP53 gene
- Somatic mutations in the TP53 gene are the most common genetic changes found in human cancer, occurring in about half of all cancers.
- In addition to the cancers described above, TP53 mutations have been identified in several types of brain tumor, colorectal cancer, a type of bone cancer called osteosarcoma, a cancer of muscle tissue called rhabdomyocarcinoma, and a cancer called adrenocortical carcinoma that affects the outer layer of the adrenal glands (small hormone-producing glands on top of each kidney).
- Most TP53 mutations change single amino acids in the p53 protein, which leads to the production of an altered version of the protein that cannot bind effectively to DNA. This defective protein can build up in the nucleus of cells and prevent them from undergoing apoptosis in response to DNA damage. The damaged cells continue to grow and divide in an unregulated way, which can lead to cancerous tumors.

Applications of p53-Based Cancer Therapy

- Because most, if not all, human cancers harbor altered p53, the concept of restoration of p53 for cancer therapy is very attractive.
- An animal model showed the reactivation of wild-type p53 to result in efficient tumor regression, including regression of lymphoma and liver carcinoma

Reactivating Mutant p53

- There is class of small molecules that reactivate the wild-type functions of mutant p53.
- **PhiKan083** is a carbazole derivative found from in silico screening of the crystal structure of p53. By binding mutated p53, PhiKan083 raises the melting temperature of mutated p53, which results in the reactivation of its function .
- **PRIMA-1** is another small molecule identified by cell-based screening which restored sequence-specific DNA binding and the active conformation of p53 .
- **CP-31398** is also a small molecule that can restore the protein folding of mutated p53 to a more natural conformation that permits a wild-type function

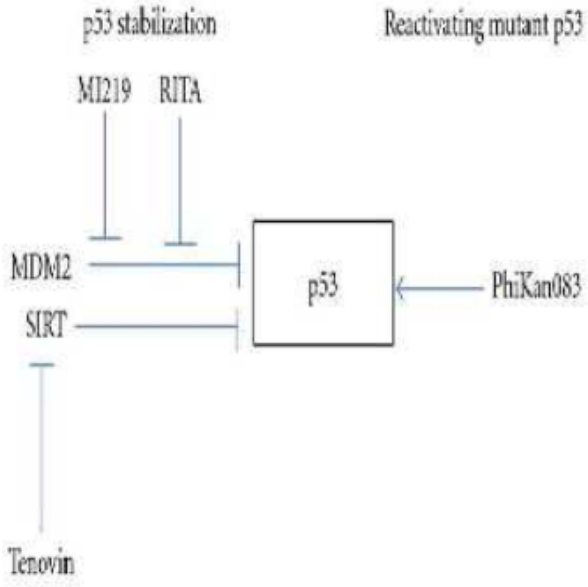
p53 Stabilization

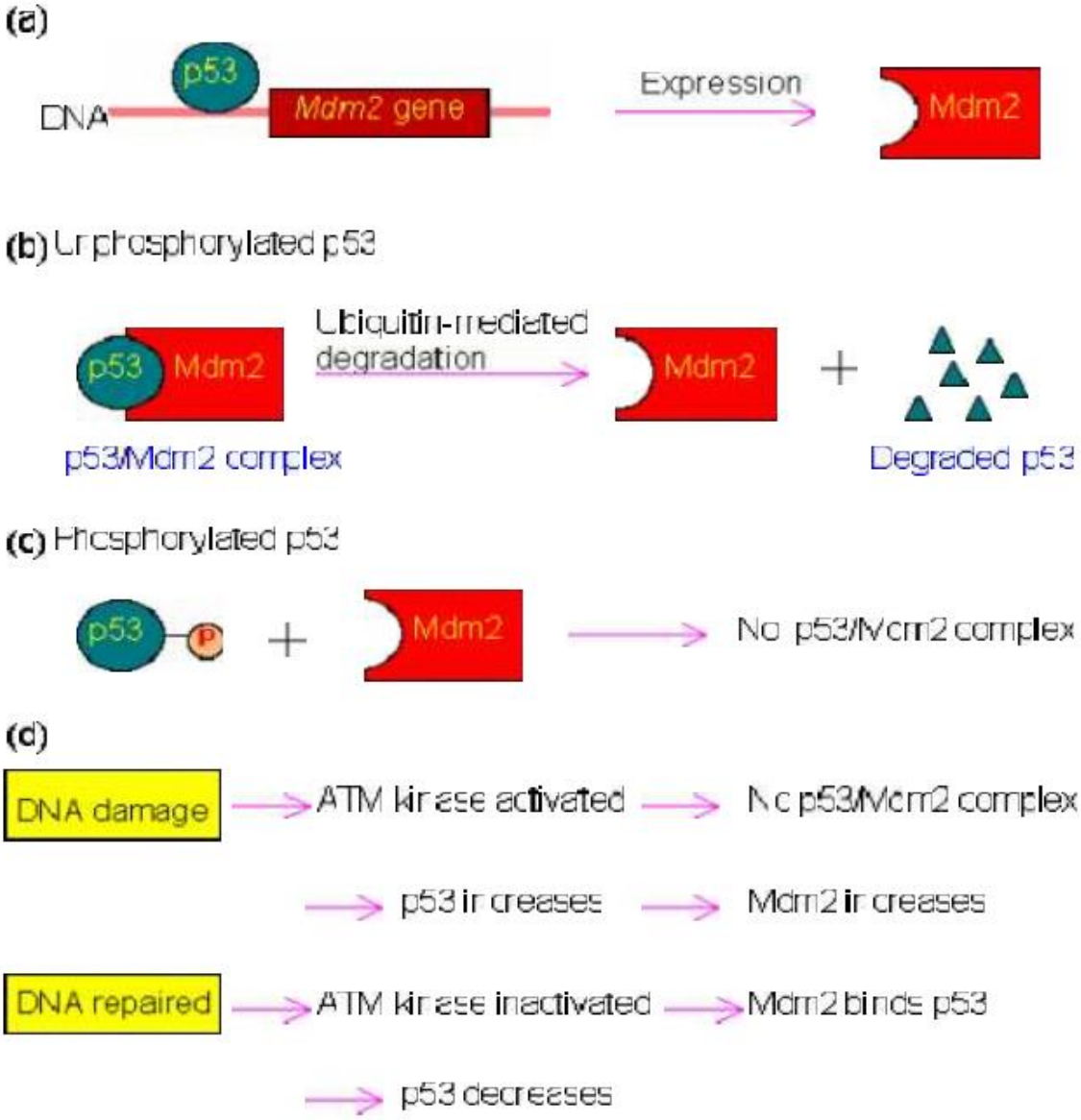
- MDM2 is an E3 ubiquitin ligase which controls p53 degradation.
- Many tumors overexpress MDM2 , even tumors without p53 mutations .

- Targeting MDM2 for p53 stabilization seems to be promising, **so many reports on targeting MDM2 or the MDM2-p53 have been published.**
- For example, the **nutlins are cis-imidazoline compounds that act as antagonists of the MDM2-p53 interaction**. Analysis of the crystal structure showed that **nutlin binds in the pocket of MDM2 to prevent the p53-MDM2 interaction**. Nutlin **can activate the p53 pathway, thereby inducing cancer cells and xenograft tumors in mice to undergo cell cycle arrest, apoptosis, and growth inhibition** .
- **MI-219 is another small molecule that inhibits the MDM2-p53 interaction**. MI-219 also activates the p53 pathway in cells with wild-type p53. Apoptosis and cell cycle arrest were observed in xenograft tumors which resulted in tumor regression .
- However, MDM2 inhibition and p53 activation in normal tissue may be harmful.

Other Classes of Drugs for p53 Stabilization

- **Tenovin** was found by a cell-based drug screen to activate p53. Tenovin acts as **an inhibitor of the protein-deacetylating activities of SirT1 and SirT2**.
- The **intraperitoneal administration of tenovin-6** has been demonstrated to induce a **regression of xenograft tumors in a mouse mode .**
- Small molecule **RITA (reactivation of p53 and induction of tumor cell apoptosis**), which binds to **p53 and inhibits the p53-MDM2 interaction** both in vitro and in vivo.
- RITA induced apoptosis in various cancer cells that retained wild type p53.





a)Expression of Mdm2 is activated by p53.
(b) Binding of p53 by **Mdm2 can trigger the degradation of p53 via the ubiquitin system**.
(c) Phosphorylation of p53 at Ser15, Thr18 or Ser20 will disrupt its binding with Mdm2. **In normal cells, these three residues are not phosphorylated, and p53 is maintained at low level by Mdm2**.

(d) *DNA damage may activate protein kinase (such as ATM, DNA-PK, or CHK2) to phosphorylate p53 at one of these three residues, thereby increasing p53 level. Since Mdm2 expression is activated by p53, the increase of p53 also increases Mdm2, but they have no effect while p53 is phosphorylated.*
After the *DNA damage is repaired, the ATM kinase is no longer active*. p53 will be quickly dephosphorylated and destroyed by the accumulated Mdm2.