Cancer

Source: NSDL, NISCAIR

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1. INTRODUCTION:

Clinical cancer can be defined as, " **a set of diseases characterized by unregulated cell growth leading to invasion of surrounding tissues and their spread (metastasis) to other parts of the body**".

Cancer is an ancient condition and was known to the early Egyptians. Sometimes the term cancer is used interchangeable with neoplasm and tumor. By definition, 'neoplasm' means new growth (known nature of growth) whereas the term tumor can be applied to both benign and malignant growths.

Carcinogenesis, the process by which cancers are generated, is a multistep mechanism resulting from the accumulation of errors in vital regulatory pathways. Mutations i.e. altered DNA bases are also required for carcinogenesis, which may involve genes that either **gain** (dominant) or **lose** (recessive) **function** as a result of mutations. Normal cells are subject to internal and external inhibitory growth signals that (to varying degrees) are lost during carcinogenesis. Cancers are clonal in origin i.e. they arise from a single cell. Although different cancers have specific characteristics but they have some common features.

A decreased rate of cell death is as important as cell proliferation in determining the size of a cancer. Also as cells differentiate they divide more slowly. Thus blocking differentiation (as in leukemia) or causing dedifferentiation (as in many cancers) increases the growth of the cancer (Fig. 1).

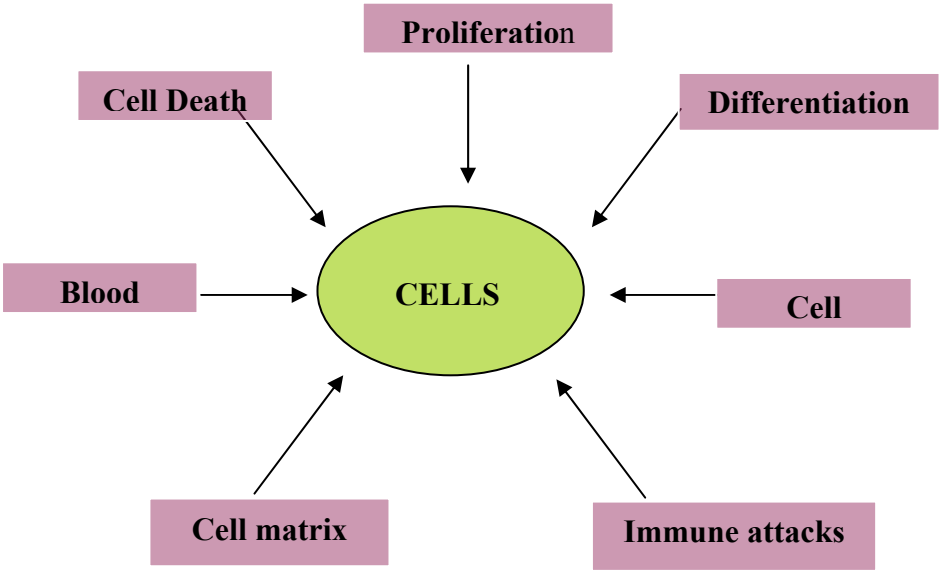


Fig. 1 Factors influencing cell behavior.

2. DEVELOPMENT AND CAUSES:

Cancer is an abnormal state in which uncontrolled proliferation of one or more cell population interferes with normal biological functioning. The proliferative changes are usually accompanied by other changes in cellular properties, including reversion to a less differentiated or developmentally more primitive state. Cancerous cells generally display several properties such as spherical morphology, expression of fetal antigens, growth-factor

independence, lack of contact inhibition, anchorage-independence and growth to high density.

Cancer has been called a "developmental disorder" (Dean, 1998) as it involves a disruption of the normal developmental program for cells, in terms of both differentiation and proliferation. It is found that some of the molecular events involved in controlling development are implicated in causing cancer. Carcinogenesis can be divided into two stages: **initiation** and **promotion**. Initiation of the cancers could be caused by external agents, yet not fully understood. **Progression** describes the additional changes which occur once the cancer growth has been initiated. Promotion can take place through various pathways in different cells. Two common features established by studies on animals during promotion are (i) altered cell proliferation and (ii) formation of new blood vessels (angiogenesis). The progression stage as demonstrated by animal experiments, in general, includes dedifferentiation, increasingly autonomous growth and aggressive behavior.

Cancerous growths can either be **benign** or **malignant**. Benign is non-cancerous and malignant is cancerous. Benign tumors are rarely life threatening, they may grow larger but do not spread to other parts of the body. They can often be removed. Malignant is cancerous. Malignant tumors can invade and destroy nearby tissues and also spread to other parts of the body. Cancer can invade the body tissues. In the early stages, the abnormally proliferating cells are usually restricted to the area in which the cancer originated. Progressive changes in the cancer cells may allow them to escape from the primary site (metastasis), and cause damage to the organism on a larger scale. A comparison of benign and malignant growth of cells is given in Table 1.

Table 1 Comparison of benign and malignant growths.

Feature	Benign	Malignant
Edges	Encapsulated	Irregular
Metastasis	No	Yes
Invasion	No	Yes
Comparison to normal	Good	Variable, often none
Growth rate	Low	High
Nuclei	Normal	Variable, irregula
Life-threatening	Unusual	Usual

2.1 MSH2 (mutS homologue 2, location; 2p22-p21) mutation triggers cancer-causing genes

The MSH2 gene is a key member of a family of about six known genes that are involved in DNA repair. Typically, when MSH2 is functioning normally, it acts like a detective, constantly surveying the cell for any mistakes to its DNA as the DNA is copied during cell replication. When it spots any mistake, it quickly repairs it. If the MSH2 gene is not functioning properly, the efficient DNA repair system slips up and lets the error become a permanent mutation in one or more of the cell's genes and in that same gene or genes in all the offspring cells. It is these successive additions of genetic defects that cause cells to proliferate wildly and become tumors.

The MSH2 gene is abnormal and mutated in cancer cells from patients who have lymphoma and leukemia. Mutations in this gene were first reported in 1993 and the MSH2 mutation triggers other cancer-causing genes to become active. When the cancer-

causing genes or oncogenes are activated by the mutated MSH2 gene, cancer results from these continuously acquire genetic mutations. A normal cell must accumulate at least 5 to 10 mutations in key genes for it to become malignant.

MSH2 is probably a common mechanism for the development of many cancers, but not that all cancers go through the same process of acquiring genetic mutations. Also never a single gene can explain all cancers although MSH2 gene seems to be a very critical gene. A mutation in this gene appears to be a very important step in the development of at least three different human cancers, evidently for its role in ovarian and breast cancer. Moreover, as scientists learn more about this gene, it is believed that it will most likely be linked to other cancers as well.

3. CAUSES OF CANCER

3.1 Chemical and radiation carcinogenesis

Chemical and radiations both cause cancer

During 1930, identification of carcinogenic chemicals and /chemical feature responsible for carcinogenicity, were major concerns of cancer research and resulted in identification and elimination of use of many industrial carcinogens.

A number of carcinogens or any agents that can induce cancer have been identified since original 18th century observation that soot causes several cancers in chimney sweeps. A common feature or characteristic of all these agents is that they damage DNA and generate changes that result in a growth advantage for the affected cell. Agents that damage DNA, either directly or indirectly through metabolic activation are classified as **Genotoxic carcinogens** and agents that exert their carcinogenic effect in other ways are **non- genotoxic carcinogens.**

3.1.1. Chemical Carcinogenesis:

There is a wide range of chemicals from simple chemical like arsenic and chromium to complex ones like aflatoxin, which are included in this category. Diet is also a major determinant of human carcinogenesis that reflects the effects of complex chemical mixtures (Table 2). For example the bladder cancer in the workers of dyestuff and rubber industries is being caused by 2-naphthylamine (β–naphthylamine), while lung cancer was associated with the chrom ium industry. Also chemicals in the manufactured items such as drinks, foods, medicines, plastics etc. are tested to determine their carcinogenic potency. Thus ensuring withdrawing /non- marketing of many potentially dangerous compounds.

Table 2 Diet and major cancers.

	Dietary factor	
Site	**Good**	**Bad**
Lung	Fruit and vegetables	
Stomach	Fruit and vegetables	salt foods
Breast	Fruit and vegetables	Fat, alcohol
Prostate	Fruit and vegetable	--
Colon	Fruit and vegetables	Meat, alcohol
Mouth, pharynx, nasopharynx	Fruit and vegetables	Alcohol, salt fish

Rather than identifying chemical that bring DNA base changes; mutations caused can be utilized to characterize the chemical nature of the carcinogenic compound. This approach of mutational spectrum analysis using molecular biology techniques is being applied to the p53 repressor genes, which provide clues to the causative events in major cancers whose aetiology is unclear.

Genotoxic carcinogens:

Four major classes of compounds exert their effects by forming covalent adducts with bases of DNA. These are:

1. The poly cyclic aromatic hydrocarbons.
2. The aromatic amines.
3. Nitrosamines, and
4. Alkylating agents.

The common characteristic of all these compounds is that they all have electrophilic groups (electron deficient) or groups that can be metabolically converted to such groups. These groups form covalent bonds with nucleophiles (electron rich) such as amino, sulphydryl and hydroxyl groups on other molecules. Nucleophiles are in proteins, RNA and DNA.

Although carcinogen interaction differ with each of these macromolecules, DNA adducts are most closely linked to carcinogenesis. Adduct formation distorts the DNA structure, so that DNA replication is disrupted. Normally it can be repaired, but if not, an inappropriate base is added (i.e. a mutation). Different carcinogens introduce different mutation. But not all mutagens are carcinogens; some are very toxic and thus lead to cell death rather a cancerous growth.

Genotoxic chemicals are activated by introduction of hydroxyl groups catalyzed by cytochrome P450 dependent enzymes (CYP) (Fig 2). Through enzymatic activation, the carcinogen is first converted to a proximate carcinogen, and then to ultimate carcinogen, which reacts with the DNA.

Environmental carcinogens taken up by body are eventually excreted, mainly in urine, but some time due to prolonged contact with bladder epithelium, they can also lead to bladder cancer as well.

Although detoxification of carcinogen may also occur by CYP-dependent hydroxylation, sulphation and glucuronide formation, but due to individual variations in genes influence carcinogenesis.

A.) Polycyclic Aromatic Hydrocarbons (PAHs)

Many of these are identified in the environment and used in experimental carcinogenesis models. Originally characterized as pyrolysis products of oils and biological materials, they are also generated in tobacco, whisky, grilled meat and by incomplete combustion of fossil fuel such a coal and petrol.

PAHs come in many configuration and some basic chemical properties only determine whether a particular PAH is carcinogenic or not. The parent compound has three aromatics fused rings (benzene), additional rings and substitutions are needed to activate the inactive phenanthrene structure to convert it into a carcinogen. The minimum requirements are (Fig.2):

1. three fused aromatic rings in the phenanthrene configuration
2. additional fused rings and/ or
3. a methyl group in the bay region.

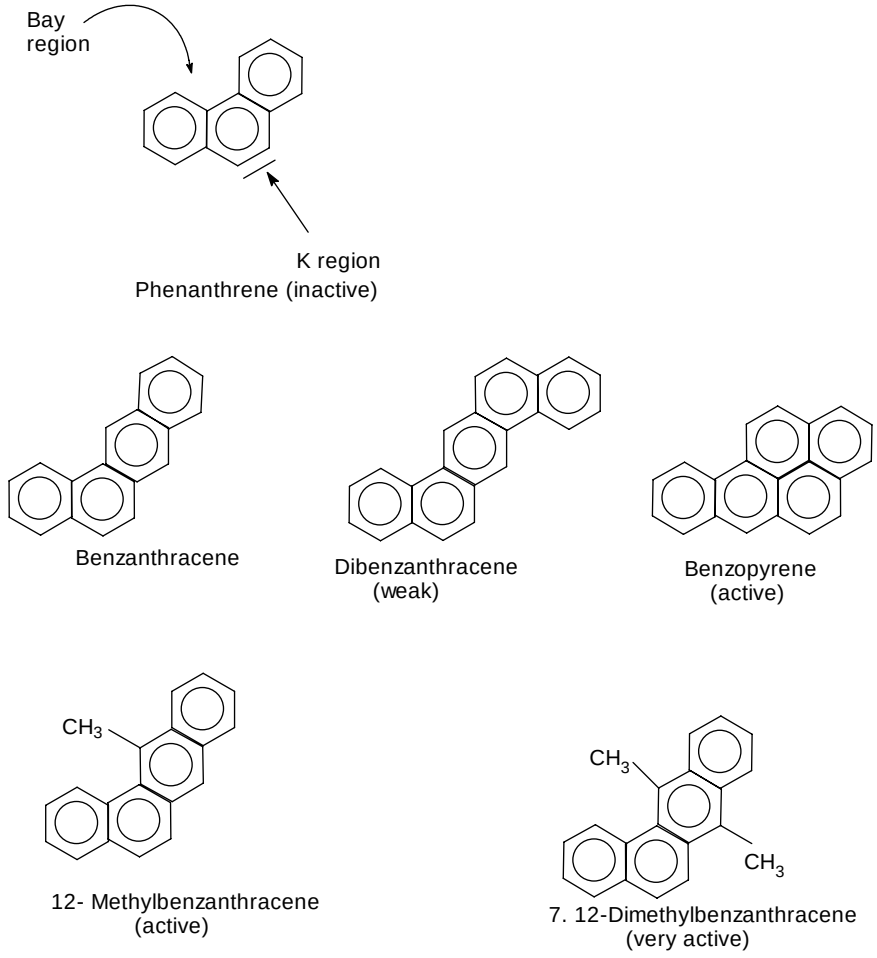


Fig.2 Carcinogenic potency of polycyclic aromatic hydrocarbons.

Two naturally occurring derivatives of the phenanthrene are the steroid hormones and cholesterol and its bile acid derivatives. Steroid hormones have been shown to be involved in the major cancers such as those of breasts, endometrium and prostate.

PHAs form adducts with purine bases, especially guanine, but only after enzymatic activation via proximate and ultimate carcinogen. For example in case of benzopyrene, CYP monooxygenaeses generate an epioxide that is converted to diol and ultimately attaches to DNA. This attachment can be to 2-amino of guanine or the 6 th amino of adenine (Fig. 3).

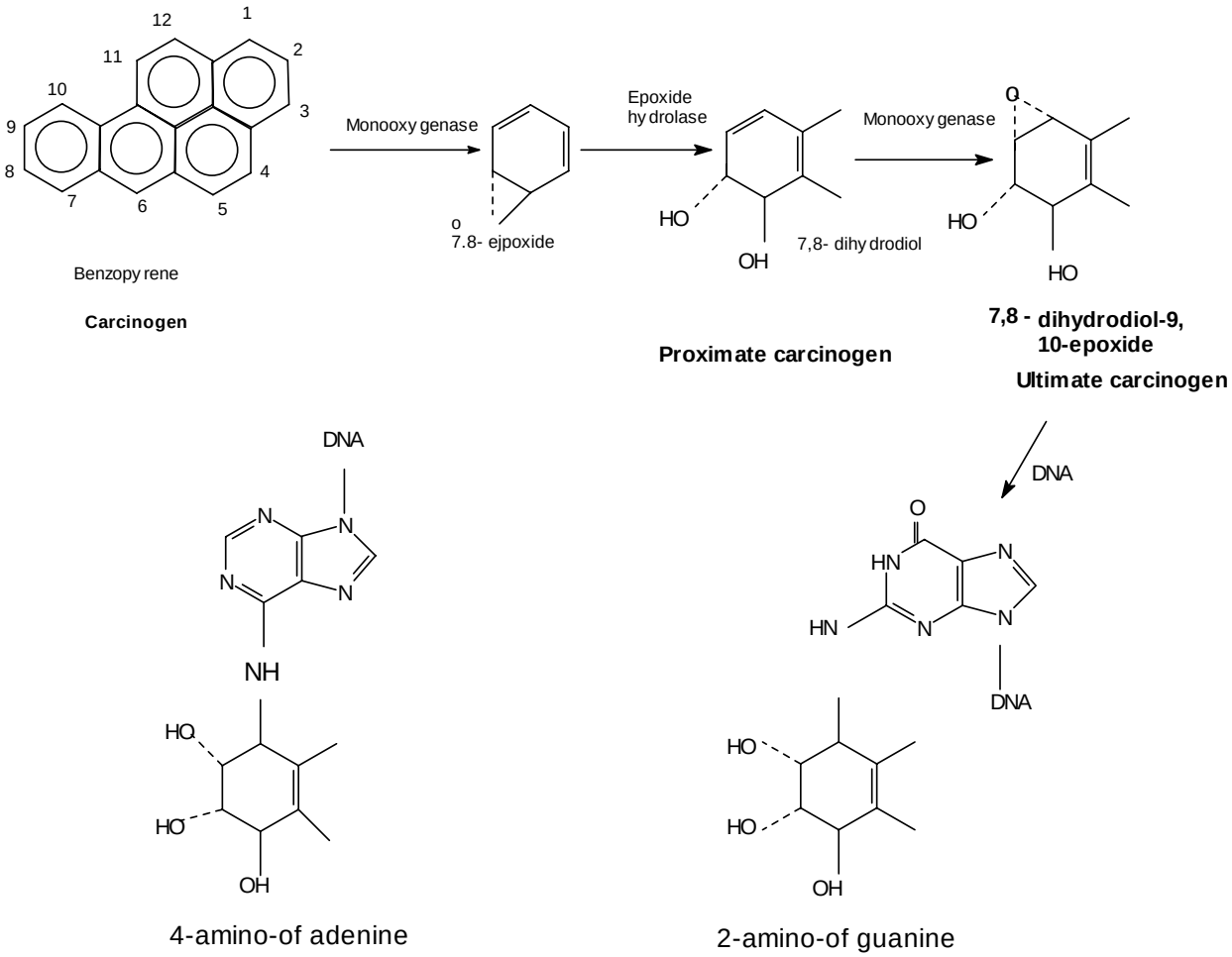


Fig.3 Activation of benzopyrene and the formation of DNA adducts.

B.) Aromatic Amines

Aromatic amines or aryl amines were identified as being hazardous through their use in the dyestuff and rubber industries. An example is 2-naphthyl amines, which has been banned because it is responsible for bladder cancer in the workers. Another chemical dismetheylaminobenzene was withdrawn when it was shown to cause liver and bladder cancer (Fig.4). The best example of cellular action of aromatic amine displayed by 2-acetyl amino fluorene (AAF), which causes multiple cancers in animals, such an bladder, liver, ear, intestine, thyroid and breast. It was originally used as an insecticide. AAF is only carcinogenic after metabolic activation through a CYP- mediated N-hydroxylase and sulphotranfarse (Fig.5). Other aromatic amines are activated by analogous mechanism through hydroxylation and ester formation at their amino groups.

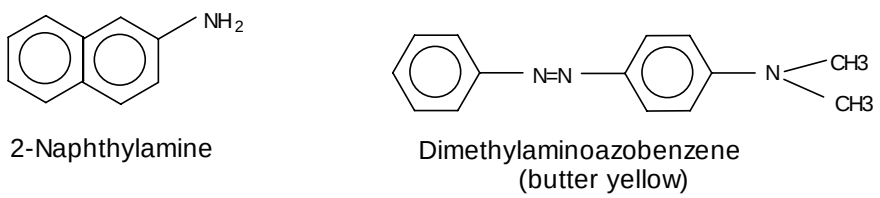


Fig.4 Carcinogenic aromatic arylamines.

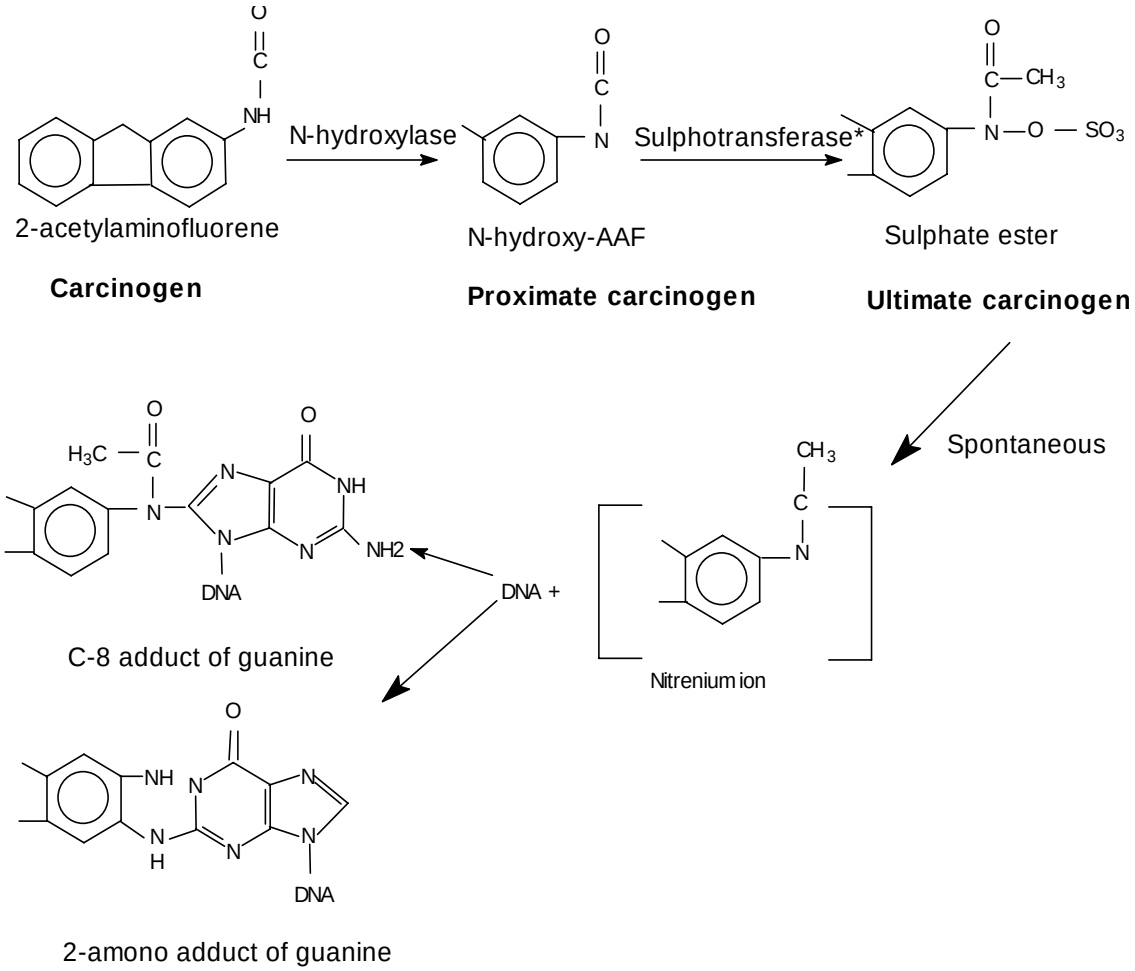


Fig.5 2-acetylaminofluorene (AAF): activation and adducts.

C.) Nitrosomines:

Nitrosamines are formed in smoked meats and fish by interaction of natural amines with nitrites added as preservatives; but they are significantly found in tobacco and its products, which contribute to lung and bladder cancer s. Chewing tobacco causes oral cancer. Enzymatically activated f orms of these f orm ultim ate carcinog en, which m ethylate guanines of DNA N- methylnitrosourea, is a nitros amines widely used for experim ental systems, as it spontaneously form s methyldiazonium ions without en zymatic activation. It is thus classified as a direct acting carcinog en with no m etabolic activation being required (Fig.6).

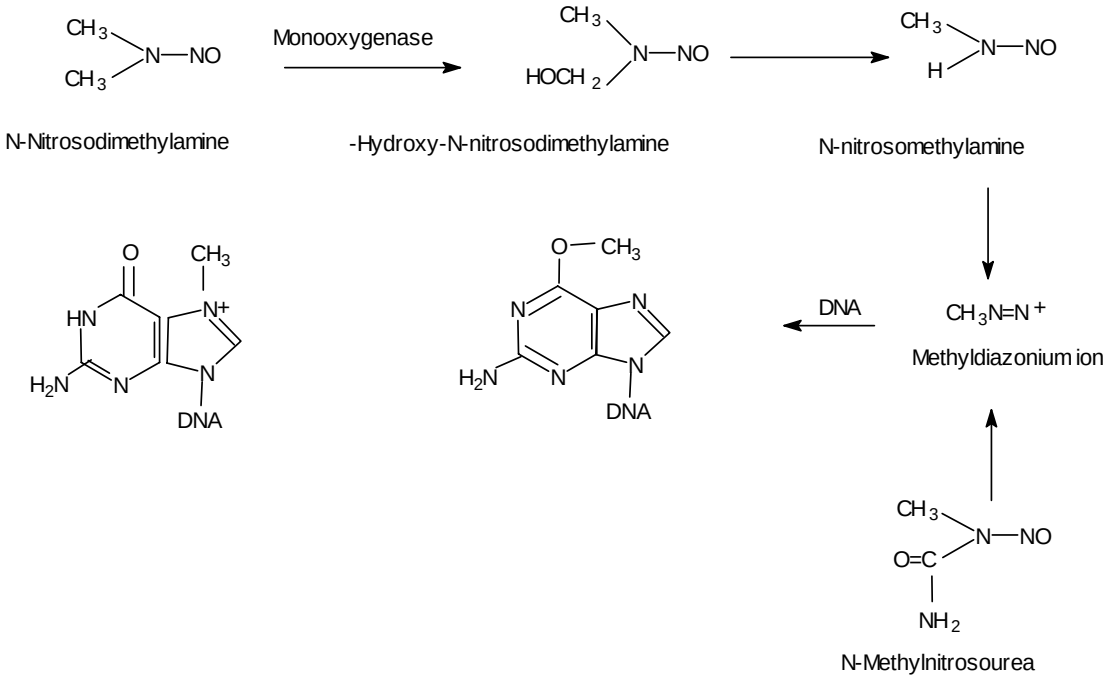


Fig.6 *N*-nitrosodimethylamine and *N*-methylnitrosourea : Activation and DNA adducts.

D.) Other alkylating agents:

Mustard gas was used during 1914-18 war, and affected soldiers s ubsequently developed higher incidences of cancer at exposed sites such as nose, bronchus and larynx. Mustard gas is a bifunctional com pound having two chlorine groups capable of reacting directly with nucleophilic am ino or hydroxyl groups as with nitrosam ines. Monofunctional alkylating agents such as vinylchloride can also be carcinogenic due to adduct form ation. Vinylchloride is used in the plastic industry as polyvinyl chloride (PVC) for products such as food wrappers.

E.) Oxidation as a cause of cancer:

Cells generate a number of reactive free radicals which can oxidize nucle ic acids, proteins and lipids and have m any of the characteristics of carcino gens. They generate structural alternations in DNA, decrease DNA repair by dam aging essential proteins and activate signal transduction pathways. The m ain source of dam aging agents is **r**eactive **o**xygen **s**pecies (ROS). The im portant ROS are supe roxide and hydroxyl radicals and hydrogen peroxide produced by electron capture (Fig.7). The cells contain protei ns and other molecules s uch as g lutathione and vitam in A, C and E, all of them are capable of inactivating ROS. Enzym atic prote ins inc lude glutathione peroxidase and catala se, which can convert hydrogen peroxide to water.

Non genotoxic carcinogens:

In this categories fall steroi d hormones, which do not damage DNA, b ut are classified as tumor promoters. These horm ones are m itogens, increase cell proliferation by binding to intracellular receptors, which are transcription factors.

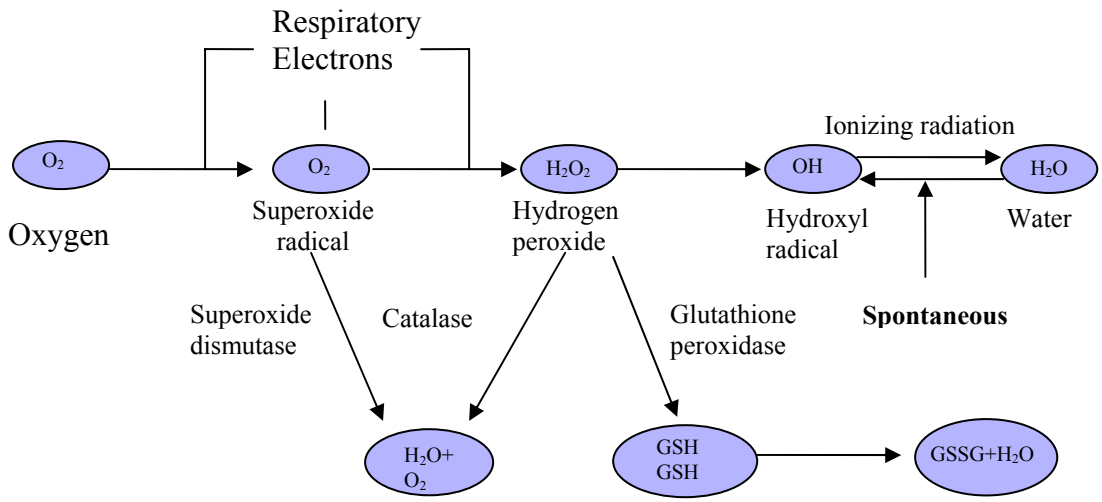


Fig.7 Production and destruction of reactive oxygen species.

3.1.2 Radiation Carcinogenesis:

Radiation is an "energy" where the rays of power depends on its source and type. Energy spectra overlap, but in descending order of energy release sequence being atomic particles > X-rays > ultraviolet (UV) light > visible light > infrared, microwaves, and electric waves. Out of these only atomic particles, X-rays and UV light have been shown to be carcinogenic. Carcinogenic form of these radiations, except UV light, generates ions in the media through which they travel and are termed as "ionizing radiation".

Ionizing radiation:

Ionizing radiation (IR), break chemical bonds and affect many molecules, but water and DNA are the principal compounds which are involved as far as cancer formation in concerned. Although physical stage of radiation carcinogenesis occurs within a fraction of a second, followed by chemical changes in which DNA bonds are broken. All these changes become apparent only after years time when cell reaches final stage.

IR produces only single and double strand breaks in the DNA, resulting in chromosome damage involving mainly deletions and rearrangements rather than point mutations generated by chemical carcinogens and UV light. Also the cells are more sensitive to IR during G2/M phases of the cell cycle whereas, early S phase is the sensitive period for chemical agents and UV light.

Ultra Violet Light:

Due to its limiting penetration capacity, it mainly affects skin especially areas exposed to sunlight. UV light spectrum can be divided into 3 regions according to its wavelength, UVA (>320 nm), UVB (290-320), and UVC (200-290). UVB is the main cause for skin carcinogenesis. Though UV radiation has lower energy and less potential for breaking chemical bonds, it can excite other molecules, making them more reactive. All photoproducts are mutagenic but the cyclobutane dimer is most important in skin carcinogenesis, as it can not be repaired quickly.

4. CANCER GENES OR ONCOGENES:

Several **oncogenes** (genes involved in tum or growth) have been defined till date using molecular cloning of the chrom osomal breakpoints including tran slocations, inversions, etc. Additional oncogenes have been identified th rough the analysis of chrom osomal regions anomalously stained (hom ogeneously stai ning regions [HSR]), representing gene amplification. Many cancers are generated by loss rather then increase of gene function an d this los s is norm ally inhibito ry inf luence, re pression in particular. Also different cancers change different signaling pathways to achieve the same uncontrolled growth.

The oncogenes were initially found out through comparison between normal and cancer cells using the technique of **transfection** (introducing foreign DNA into intact culture cells). For example the carcinogenicity of *ras* gene from bladder cancer was identified by this technique. The molecular pathogenesis of human cancer is due to structural and/or functional alterations of specific genes whose normal function is to control cellular growth and differentiation or, in different terms, cell birth and cell death. Transf ection experiments were also used to test the effect of s pecific m utations on gene func tions. One m ilestone was the use o f DNA transfection technique that helped to clar ify the cellu lar origin of the "viral oncogene s." The latter were previously characterized as the specific genetic elements capable of conferring the tumorigenic properties to the RNA tum or viruse s also known as retroviruses. Further, the transfection technique led to the identification of cellular transforming genes that do not have a viral counterpart. Besides th e source of their or iginal identificati on, viral or cellular genome, these transforming genetic elem ents have been designated as protooncogene in their normal phys iological version and oncogene when altered in cancer. Thus an oncogenes can be defined as; **"any gene that encodes a protein- able to transfor m cell in culture or to induce can cer in animals".** T hey are d erived from normal cellular genes (i.e. protooncogene) whose products prom ote a bnormal/uncontrolled cell proliferation. Conversion or activation of a protooncogene into an oncogene generally involves a gain of function mutation. Also gene modification results in either qualitative or quantitative changes in gene expression (Fig.8). Different m echanisms which help in activation of a protooncogene into an oncogene are:

1. Point mutations
2. Chromosomal translocations
3. Amplification of a gene.

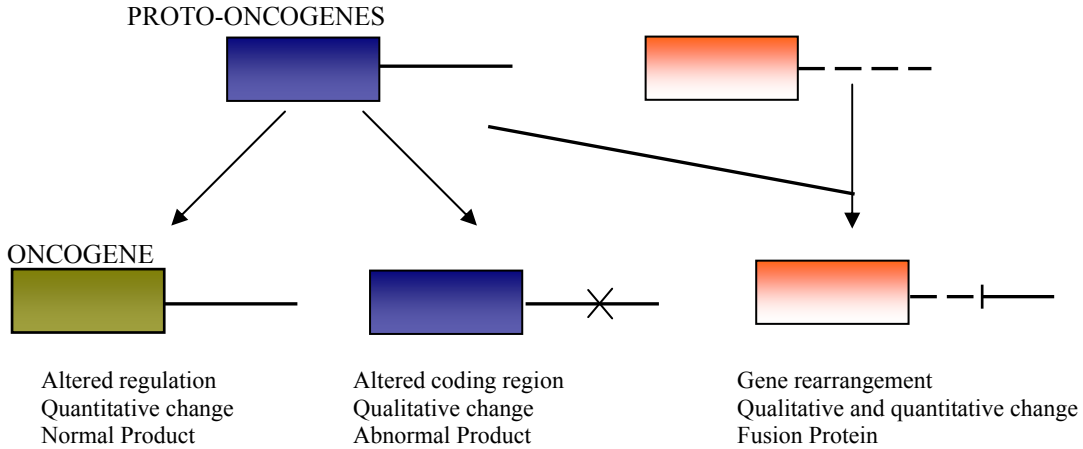


Fig.8 Oncogene activation.

4.1. Loss of gene function can be carcinogenic:

Several tumor-suppressor genes have been identified, which encode for proteins that inhibit cell proliferation. These proteins are also involved in the regulation of cell proliferation and death. Different terms such as 'repressor' or 'anti-oncogene', 'recessive' and 'loss of function' have been used to describe this phenomenon. There are some major classes of proteins which are recognized as being encoded by tumor-suppressor genes:

1. Intracellular proteins that regulate or inhibit specific stage of cell cycle (e.g. p16 and Rb, Fig. 9)
2. Receptors or signal transducer or developmental signal that inhibits cell proliferation (e.g. TGFα, the hedgehog receptor patched).
3. Checkpoint control proteins.

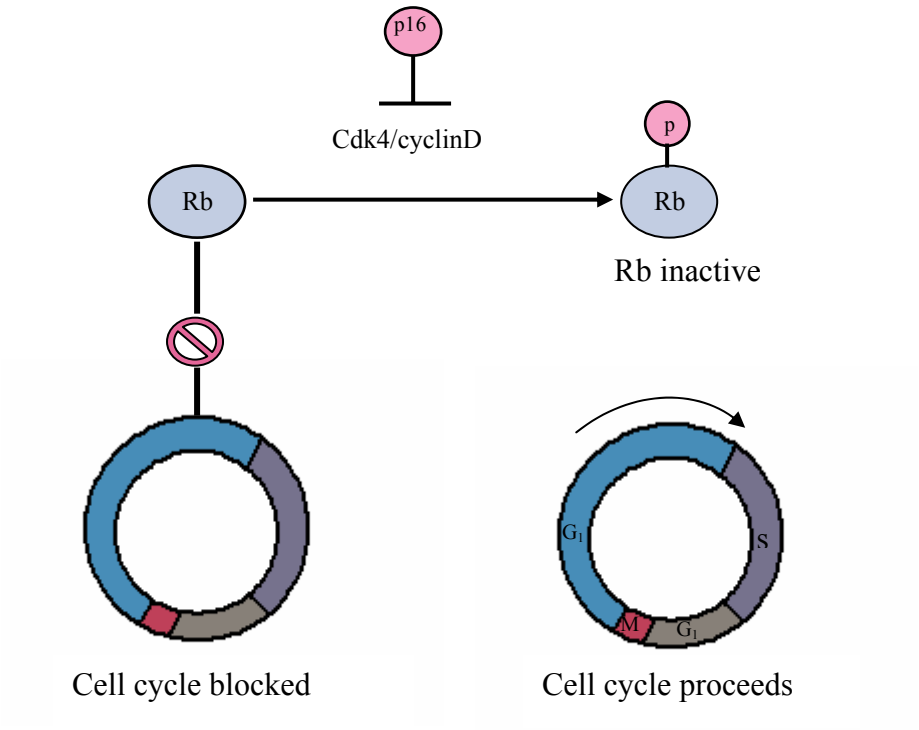


Fig.9 Role of Rb and p16 in cell cycle inhibition.

4.2. Gene amplification can convert a proto-oncogene into oncogene:

The oncogene encodes an 'oncoprotein' that differs from the normal proto-oncogene product. The localized amplification of DNA to produce many copies of a given region is also a common genetic change seen in tumors. This can be in two forms i.e. either duplicated DNA may be randomly organized at a single site on a chromosome, or it may be found as small, independent chromosome like structure. The example of gene amplification causing tumors is seen in genes such as *N-myc* gene and its neighbor *DDX1* that are amplified in neuroblastoma. Such gene amplifications can be establishes by microarray technique.

5. TUMOR VIRUSES:

Peyton Rous (1911) initially recognized that a virus can cause cancer. Later on, Rouse Sarcoma virus (RVS) was shown to be retrovirus (whose RNA genome is reverse transcribed into DNA). In addition to the normal genes, RSV contains *v-src* and it was shown through mutation studies that only *v-src* was required for tumor formation.

In 1970, scientists found that normal cell from chickens and other species also contain the closely related gene, which is a protooncogene and is designated as *c-src* gene. RSV and other oncogenes carrying viruses are thought to have arisen by incorporating, or transducing a normal cellular protooncogene into their genome. Later, mutations in these genes can convert them into dominantly acting oncogenes, which can induce cell proliferation in the presence of normal *c-src* protooncogene. These types of viruses are also known as transducing viruses. As the genome of RSV contains *v-src* oncogene, it can induce cancerous growth within days. In contrast to RSV, there are many other viruses which take months or years to induce tumor growth. The reason for their slow action, in comparison to transducing viruses, is the absence of an oncogene. All slow acting or 'long latency' retroviruses appear to cause cancer by integrating into the host cell DNA into or near a cellular protooncogene and activating its expression.

Classification of Tumor viruses:

Six families of viruses, causing cancer in human, can be broadly classified as (Table 3):

1. DNA tumor viruses
2. RNA tumor viruses (Retro viruses)

5.1. DNA viruses constitute five families and they have DNA genomes.

DNA viruses propagate into host cells in two different ways i.e. in permissive cells, viral genome is completely expressed and leads to viral replication and thus cell lysis and cell death.

In case of non permissive cells, DNA genome of viruses integrates into the cell chromosomes at random sites and only part of it is expressed. In this case, viral structural proteins are not expressed and thus no lysis and no viral progeny are released. Tumor viruses have played a critical role in cancer research by serving as models to study mechanisms of protooncogene activation.

5.1.1. Family: Papovaviridae, e.g. Papilloma and Polyoma viruses.

These are small DNA viruses (genome ~8 kb) and can induce both benign and malignant tumors in humans as well as other animals. They are wart-causing viruses and also cause human neoplasm and other natural cancers. About 60 different types of human papilloma viruses, which have been identified, infect epithelial cells of different tissues. Papilloma

viruses are also associated with human penile, uterine and cervical carcinomas. Genital warts can convert to carcinomas.

Table 3 Viral carcinogenesis.

Virus	Gene	Function	Compartment
RNA			
Rous sarcoma	$PP60^{SRC}$	Tyrosine kinase }	Cytoplasm, membranes
Rat sarcoma	ras	GTPase }	
Erythroblastosis	erbA	Thyroid hormone receptor	Nucleus
Feline osteosarcoma	fos	Transcription factor	Nucleus
Simian sarcoma	sis	Platelet-derived Growth factor	Secreted
Mouse mammary tumour	None	Insertional mutagenesis	-
DNA			
Human papilloma	E6, E7	Bind repressors (p53,Rb)	Nucleus
Adenovirus	E1A, E1B	Bind repressors (p53,Rb)	Nucleus
Simian virus 40	Large T antigen	Bind repressors (p53,Rb)	Nucleus
Epstein-Barr	BZLFI, EBNA5	Binds p53; rearranges *myc* genes?	Nucleus
Hepatitis B	HBX	Binds p53	Nucleus

There are about 51 types of papilloma viruses, but not all of them cause cancers. Papillomo viruses cause 16% of female cancers and in all 10% of all cancers. Human papilloma viruses (HPM), cause cancerous growth which involves expression of two genes, E6 and E7. The E6 and E7 proteins act analogously to SV40 T antigen by interfering with the function of the cellular Rb. and p53 proteins. Specifically, E7 binds to Rb and E6 stimulates the degradation of p53 by ubuquitin –mediated proteolysis (Fig. 10).

Simian virus 40 and Polyoma Viruses

Simian virus 40:

SV40, monkey polyoma virus causes sarcomas in Juvenile hamsters. This is one of the best studied tumor viruses. Though not a causative agent for human cancers it has served as a model system for understanding the molecular basis of cancerous transformation of cells. This has been possible due to the availability of good cell culture assays for virus replication and cell transformation and also these viruses can be easily handled due to their genome size being small (~5kb).

Polyoma virus was originally isolated from AK mice and is fully permissive for replication in mouse cells. It causes leukemia in mice and hamsters. Both SV40 and polyoma viruses do not induce tumors or transform cells in monkeys and mice. These cells are permissive for these viruses and infection leads to virus replication, cell lyses and killing of cell and therefore doesn't lead to the transformation. Later, they can infect other non- permissive cells (no viral replication) where viral genome integrates into cellular DNA and expressed viral genes results in transformation of infected cells. SV40 and polyoma virus genes responsible cell transformation, their RNAs, and transforming potentials of individual viral genes, viral mutants unable to induce transformation, have been isolated and studied in detail by molecular analysis.

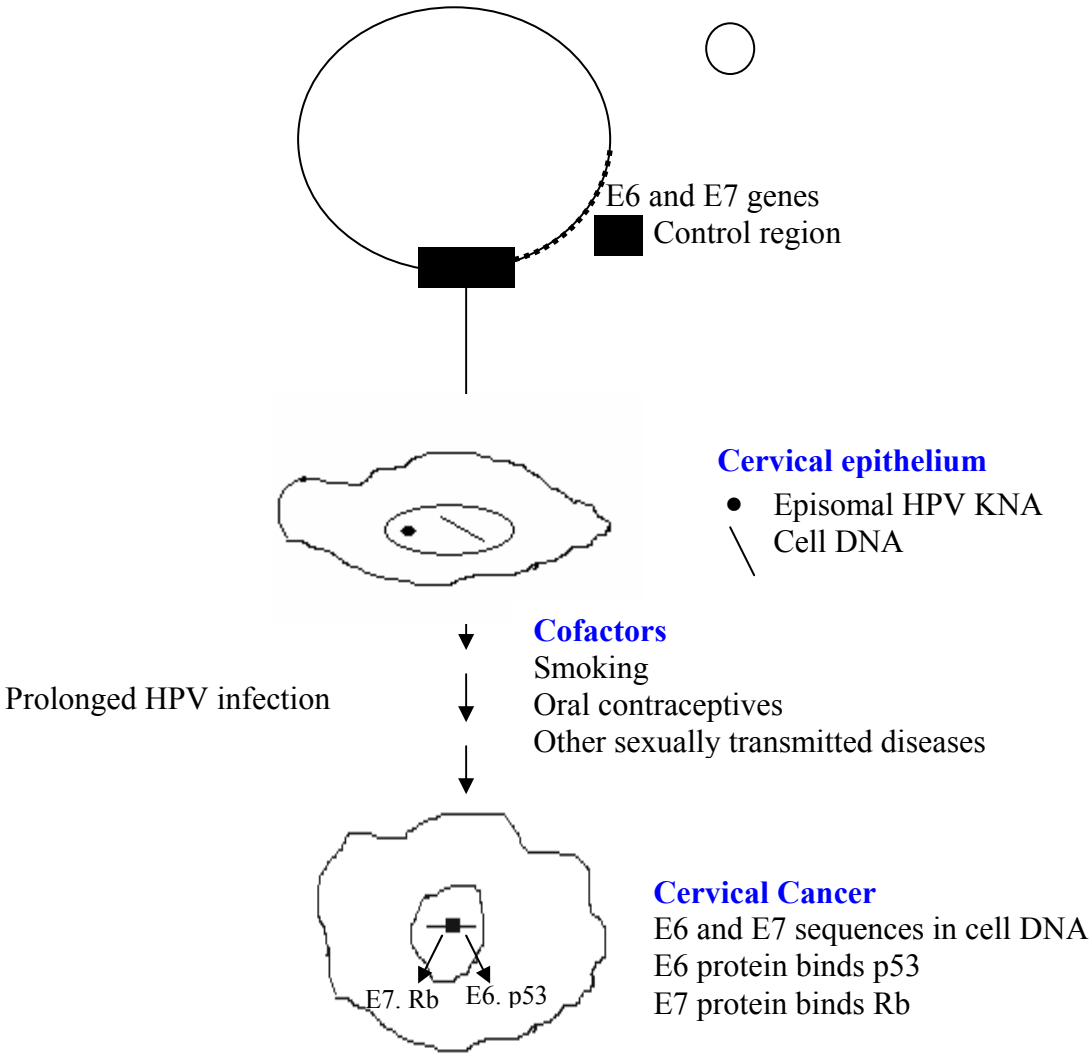


Fig. 10 Human papilloma virus and cervical cancer.

The genomes of SV40 a nd polyoma virus have b een divided into early and late regions. Early region expresses immediat ely after infection whereas, la te region is not expressed until viral DNA replication begins. Early region includes genes required for synthesis of viral DNA, whereas, late region has genes encoding structural components of virus particle.

Early region of SV40 encodes 2 protein i.e. small and lar ge T antige ns, of ~17 Kd and 94Kb, respectively. Polyom a virus likewise enc odes sm all and large T antigen a s cell a third protein of ~55 Kd, de signated as m iddle T. Large T in SV40 and m iddle T i n polyoma virus are responsible for transformation of a cell to the cancerous state (Fig.13).

5.1.2. Family: Adenoviridae (e.g. Adenoviruses)

This family includes highly oncogenic DNA viruses with genome size in the range ~35 kb. These viruses are not associated with natural cancers in hu mans or any other anim als but they are important m odels in experim ental cancer biology. In these, only a portion of the viral genome is in tegrated into host genom e. They are lytic in ce lls of their natu ral host species and can induce cancer in non-permissive hosts. Expression of two early genes E1A

and E1B is required for replication of viru s (E1A region contains the oncogenes that encodes for several T antigens) binds to the product of Rb gene, and E 1B product binds with p53. This polyoma and adenoviruses, SV40, all seem to cause cell transformation in a similar manner. They alter cell cycle regulation by interfering with the activities of Rb and p53.

5.1.3. Family: Herpesviridae. (E.g. Herpes viruses)

They are the m ost complex, enveloped DNA viruses with genom e size of 100 to 200 kb. They are highly tum origenic in an imals. These have been im plicated in m any hum an neoplasms. Several herpes viruses induce tu mors in anim al species , includ ing frogs, chickens and m onkeys. Herpes viruses exist primarily as episom es in the cell without integrating into host genom e. They m ay employ a hit and run m echanism of oncogenesis, by the time tumor arises, no trace of the virus is found. They are able to do so, presum ably by causing chromosomal breakage or other damages.

Esptein-Barr virus:

This is m ost strongly associated with cancer and is causally associated with cancers like, Burkitt`s lymphom a in tropics (region of Africa), Nasophar yngeal cancer in other areas (common i n China and SE Asia). B-cell lymphomas in AIDS patients or other immunosuppressed individuals (as in organ transplantation), H odgkin's lym phoma, EBV has been found associated with m ost of Hodgki n's lymphomas (40% of affected patients). EBV is able to transform hum an B lym phocytes in culture as we ll. Though several viral genes responsible for inducing transformation of lymphocytes have been identified but still the m olecular Biology of EBV replication and transformation is still not fully understood partly because of its complex genome. EBV causes infectious mononucleosis but as to why this virus causes benign tumors in some and malignant in others is still a mystery.

Human Cytomegalovirus:

Initially associated with Kaposis's sa rcoma, but now thought probably caused by a newly discovered herpes virus, human herpes virus 8.

Herpes simplex II:

Associated with cerv ical cancer in epidem iological studies but now evid ences for papillma virus being the causative of cervical cancer are available.

5.1.4. Family: Hepadanoviridae e.g Hepatitis B viruses

This is the sm allest DNA virus with a genom e size of ~3Kb. It inf ects specifically liver cells of several species, incl uding squirrels, wood chucks, ducks, and hum ans. Hepatitis B is a vast public health problem and hepato cellular Carc inoma (HCC), which is one of the world's m ost comm on cancers, is caused by HBV. Although a DNA virus, it is m ore similar to the RNA tumor viruses (oncoran viruses), in its mode of replication. Infection by Hepatitis B virus results in acute liver damage. About 5 to 10% of acute infection cases are not resolved and chron ic infection of liner develops. HBV infections are more common in parts of Asia and Africa (~ a m illion cases of liver cases annually). Although HBV are causative agents of a m ajor human cancer, the mechanism is still not well understood. It is assumed that the tum ors result from expression of a viral gene (the *X* gene) that stimulates transcription of cellula r protooncogene which in turn drive cell prolif eration. There is a correlation between HBsAg (hep atitis B virus surface an tigen), chron ic carrier and the

incidence of hepatocellular carcinoma (HCC). 51% of deaths of HBsAg carriers are caused by liver cirrhosis or HCC compared to 2% of the general population.

5.2. RETROVIRUSES (RNA tumor viruses)

These viruses have RNA in their genome thus are different from DNA viruses but have similar mode of integration into host genome. **RNA must be copied to DNA** prior to integration into host cell genome by the process of reverse transcription. Members of retroviruses cause cancer in variety of animal species, including humans.

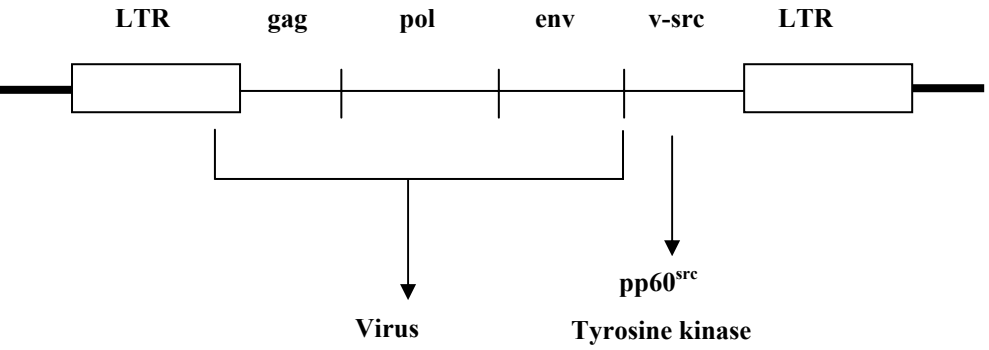
Retrovirus structure:

Outer envelope comes from the host cell plasma membrane, coat proteins (surface antigens) are encoded by *env* gene. Primary product is cleaved so that there is more than one surface glycoprotein in the mature virus. Inside the membrane is an icosahedral capsid containing proteins encoded by the *gag* gene (Group-specific antigen). *Gag* encoded proteins also code genomic RNA.

There are two molecules of genomic RNA per virus particle with 5` cap and a 3` poly A sequence. Thus, virus is diploid. The RNA is plus sense (same sense as m RNA). About 10 copies of reverse transcriptase are encoded by *pol* gene within the mature virus. The *pol* gene also code for several other functions. The *Pol* gene products are:

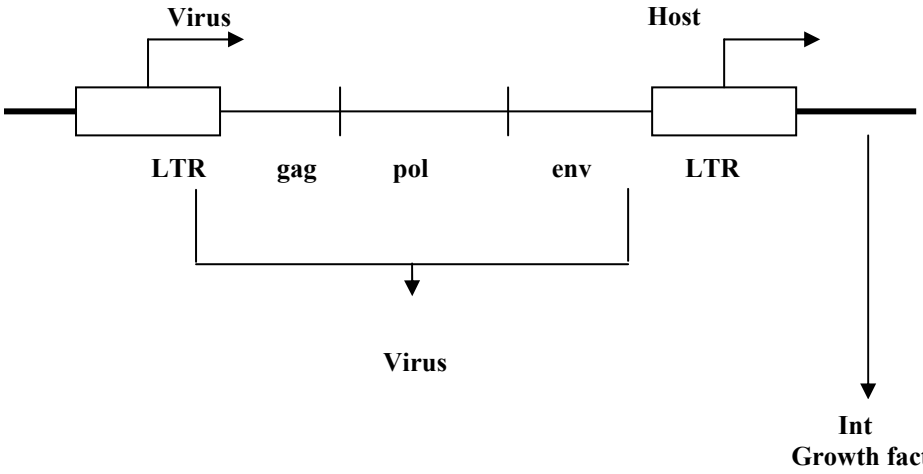
(a) Reverse transcriptase, (b) Integrase (integrates the viral genome into the host genome), (c) RNase H (cleaves the RNA as the DNA is transcribed so that transcriptase can make the second complementary stand of DNA) and (d) Protease (cleaves the polyproteins translated from mRNA from the *gag* gene and the *pol* gene itself).

RNA carcinogenesis proceeds in one of the two ways (Fig.11): by providing an oncogene or insertional mutagenesis, in which regulatory viral sequences alter host gene activity. In humans, insertional mutagenesis is unknown although it is common cause of animal cancers.



Virus provides an oncogene: Rous sarcoma virus and v-src. Regulation provided by long terminal repeat (LTR)

Regulation of transcription



Insertional mutagenesis: mouse mammary tumor virus. Upregulation of a host growth factor gene

Fig.11 Viral Carcinogenesis.

6. TUMOR SUPPRESSOR GENES:

Chromosome deletions and their detection have been instrum ental for the identification and cloning of a second class of cancer-associated genes, **the tumor suppressors**. Contrary to the oncogenes that are activated by dom inant mutations and prom ote cell growth, tum or suppressor genes act in the norm al cell as **negative controllers** of cell growth and are inactive in tum or cells. In gene ral, therefore, the m utations inactivating tum or suppressor genes are of the recessive type.

Tumor suppressor genes act as negative control of cell growth and are inactive in tumor cells. They play a very critical role in cell division cycle. If DNA damage is detected in the cell, the tumor suppressor gen e can stop the cell from multiplying until the DN A damage is repaired or they can even stim ulate the cell with DNA dam age to co mmit "cell suicide." T hus, the tumor suppressor genes code for "S TOP" signals th at instruct the cell to leave cell cycle and stop dividing.

In tumor cells these genes are either lost or inactivated and hence the negative control of the cell proliferation is lost. This leads to abnormal cell growth and proliferation i.e. the cancerous growth.

Tumor suppressor genes were identified using somatic cell hybridization experiments, initiated by Henry Harris and his colleagues in 1969. In these experiments, a normal cell is fused with a tumor cell, resulting into hybrid cells containing chromosomes from both parents (Fig 12). In most cases, it was observed that in animals the hybrid cells were not able to form tumors. Therefore, it was concluded that genes derived from normal cell parent inhibited (suppressed) tumor development.

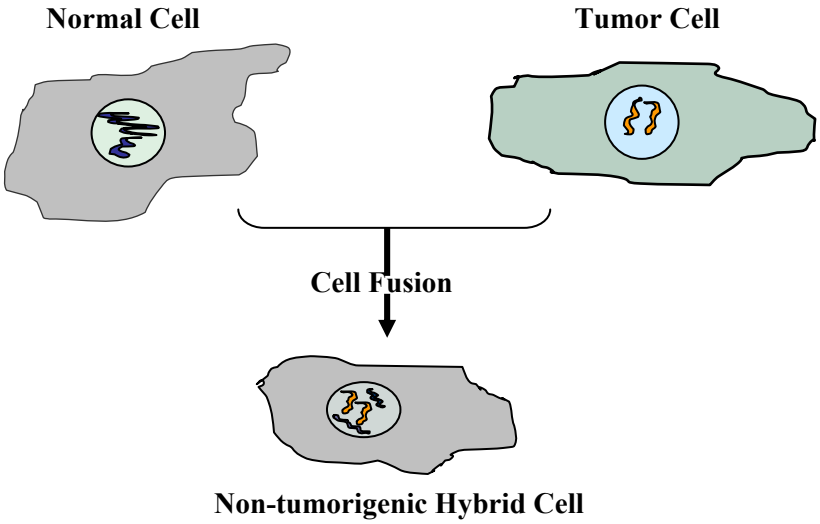


Fig.12 Hybrids cells, obtained from fusion of a normal cell with tumor cell, are usually non-tumorigenic.

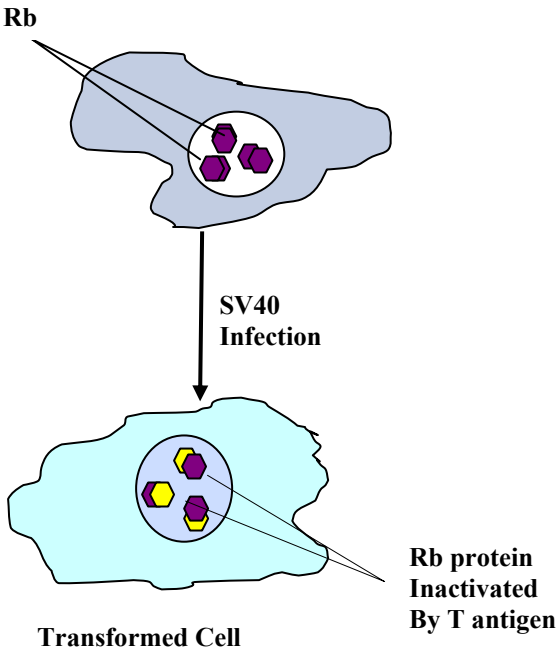


Fig.13 Interaction of Rb and oncogene proteins of DNA tumor viruses.

The first tumor suppressor gene identified was from studies on retinoblastoma, a rare childhood eye tumor. Retinoblastoma is transmitted as a dominant trait but inheritance of susceptibility genes in not enough for causing cancer. This shows that tumor development requires some additional factors. In 1971, Alferd Knudson proposed that the development of retinoblastoma requires two mutations, corresponding to the loss of both functional copies of the tumor susceptibility gene, (Rb, tumor suppressor gene). The product of the Rb gene is a nucleus-located protein of 105 Kd. Thus, in heterozygote condition tumor can be suppressed but in homozygote, where no functional Rb allele is present, tumor can not be suppressed. However, the non inherited retinoblastoma are rare, as its development requires two independent somatic mutations to inactivate both normal copies of Rb in the same cell. Also Rb protein is a key target for the oncogene protein of several DNA tumor viruses, e.g. SV40, adenoviruses, and human papilloma viruses, which bind to Rb and inhibits its activity. Thus, transformation by these viruses also results from inactivation of Rb at protein level. Rb product regulates cell cycle progression at same point as that affected by cyclin D1. It inhibits passage through G1 by repressing transcription of a number of genes involved in cell cycle progression and DNA synthesis.

6.1. p 53:

p53 named for its size (53kda), was discovered as a cellular nuclear phosphoprotein associated with the SV40 large T antigen. The gene product regulates both cell cycle progression and apoptosis. It has been found to be inactivated in a wide range of human cancers e.g. leukemia, lymphomas, sarcomas, brain tumors and carcinomas of breast, colon and lung etc. There is a correlation between the abundance of p53 and the oncogenic activity of the tumor virus DNA damage which leads to rapid induction of p53, which in turn

activates transcription of the Cdk inhibitor p21. This in turn blocks cell cycle progression, by acting as an inhibitor of Cdk/ cyclin complexes and by inhibiting DNA replication by binding to PCNA (proliferating cell nuclear antigen).

Like Rb, p53 also forms a complex with SV40 large T antigen, and E1B transforming protein of adenovirus and E6 protein of human papilloma viruses. Complexing with these tumor antigens leads to increased p53 stability. Phosphorylation regulates the activity of p53. Levels of p53 are low after mitosis but it increases during G1 and during S phase, the protein becomes phosphorylated by the M-phase cyclin-CDK complex of the cell cycle and also by casein kinase II (CKII).

Also, p53 protein has been shown to block the binding of DNA polymerase to SV40 large T, blocking replication of SV 40 DNA. Another important function of p53 is to instruct a cell with DNA damage to commit suicide. Otherwise there damaged cells would continue to divide and accumulate mutations which might allow the cell to escape from normal controls and to form a tumor.

p53 also plays an important rote in apoptosis is induced by DNA damage. Cells lacking p53 fail to undergo apoptosis, in response and agents that damage DNA, in cluding radiation and drugs used in cancer chemotherapy. These effects of p53 inactivation on cell survived are thought to be the reason for high p53 mutations in human cancers.

6.2. Neuro fibronatosis Type 1 (Nf1):

The protein encoded by NF1 tumor suppressor gene is 2485 amino acid long and is 220 kd. It shares a striking homology to *ras* GAP. NF1 protein is called neurofibromis and its expression is shown in all tissues. Development of benign neurofibromas versus malignant neurofibrosarcomas may be due to the inactivation of one NF1 allele and both alleles, respectively. However, some other changes are also indicated in the genesis of neurofibrosarcomas e.g. there is a consistent loss of genetic material on the short arm of chromosome 17 in case of neurofibrosarcomas but not in neurofibromas. Protein encoded by Nf1 tumor suppressor gene is a good example of antagonism between oncogene products and tumor suppressor gene products.

The NF1 protein down-regulates Ras prooncogene protein by acting as a GTPase activating protein. High levels of active GTP- bound Ras are therefore present in tumor cells in which NF1 has been inactivated, which in turn stimulates cell proliferation as a result of deregulation of the Ras signaling pathway.

6.3. Familial Adenomatosis Polypsis (FAP)

Mutations in adenomatous polyposis coli (APC) gene are responsible for colorectal cancer (non-inherited) as well as familial adenomatous polyposis (FAP) (inherited). APC exhibits a dominant pattern of inheritance. Individuals with this dominant mutation develop multiple colonic polyps, which arise during 20-30 years of life and some of them inevitably progress to malignancy. FAP adenomas result as a consequence of loss of function mutation in APC gene. Till now more than 120 germline and somatic mutations have been identified out of which majority of these mutations lead to COOH-terminal truncation of APC and emaciated polye protein. The protein encoded by APC binds β catener (α family of protein that interacts with the cytoplasm portion of the Catharine which links actin filaments to transmembrane protein).

7. MOLECULAR BIOLOGY AND CANCER

Prevention and early detection are critical determinants of the disease. Early detection using molecular detecting kits can lead to identification of individuals with inherit susceptibility to

cancer development, e.g. early detection of premalignant stages of colon cancer that remain localized is very high ~90%. However, survival rates drop to ~50% if the cancer has spread to other tissues and less than 10 %, if it has come to metastatic stage. Completion and deciphering of human genome in recant years have opened up an exciting new era of research on cancer prevention, diagnosis and treatment. New drugs are being designed to target specific characteristics of cancerous cells at the molecular level. This includes genetic mutations, factors in gene expression, alternations in signaling pathways and structural changes in the proteins involved in cancer growth of the cells.

Monitoring and early detection also help in family planning decision and more effective treatment of many types of Cancers e.g. patients with familial adenomatous polyposis (inherited mutation of the APC gene, tumor suppressor) develop hundreds of adenomas within the these people can be removed before it progresses to malignancy.

STRATEGIES FOR MOLECULAR TARGETING

Different strategies are being designed and put to use for combating cancer at molecular level, these are:

(1) Diversion of a cancerous cell towards a more normal cell growth patterns,

(2) Activate /stimulate immunosystem of the body to reject cancerous cells /destroy them,

(3) Inducing apoptosis in cancerous cells,

(4) Stopping cell from repeated replication of itself,

(5) Inhibiting cell to use surrounding cells to support its own growth e.g. angiogenesis.

Drugs developed so far using traditional parameters and strategies are having a disadvantage that they attack both cancerous and healthy cells. This leads to short and long-term side effects. Also the individual responses to conventional drugs vary. In these situations molecularly targeted drugs/therapies hold the promise of being more highly selective, significantly reducing the side effects as well.

Infact, the molecularly targeted drugs are being designed in such a way that they interfere with specific biological process needed for cancerous growth with minimal toxicity. These developments in the ongoing molecular biology research in cancer treatment will soon be able to provide specifically tailored molecular treatments for the individual patients. These molecularly targeted agents will ultimately be able to disrupt multiple molecular pathways and thus slowing down or halt disease progression.

These molecularly targeted agents are categorized into two major classes:

1. Small molecule compounds which modulate gene expression and protein essential for cancerous growth of the cells.
2. Biological agents derived from components of the immune system which can activate immune system to block cancerous growth.

It has been already found out that genes may allow to predict results of a treatment going on for any individuals e.g. in immunotherapy. Recent studies have shown that a set of genes which regulate T-cell response are over expressed in metastatic lesions.

Molecular designing of drugs or any treatment is based on the diagnosis. Traditional diagnostic categories include anatomical location and examination of cancer cells under microscope. But the disadvantage of such diagnostic methods is that they fail to differentiate

between molecularly distinct forms of cancer. Therefore, most of the time patients fail to respond to standard treatments with conventional agents. Molecular diagnosis in these situations has distinct advantage as it can classify cancer on the basis of molecular characteristics that would provide profound clinical benefits.

Molecular signature can be developed that can be basis of clinical diagnostic tests. These will help in ascertaining beforehand the likelihood of cure by surgery alone. In addition, molecular profiling can be used to discover novel molecular targets for the development and designing of drugs. Further, in case of non-Hodgkin's lymphoma also known as diffuse large B-cell lymphoma (DLBCL), the standard chemotherapy works only in ~40% of patients but reclassification of DLBCL based on the molecular characteristics divides this into two major subclasses and make treatment more specific. Also identification of patterns of genes that are active in tumor cells leads to the development of more targeted treatment and delivery of more accurate prognosis to patients.

Early Detection Using Molecular Biology Tools

Earlier cancers were detected mostly at a much advanced stage, when invasion and metastasis have already occurred. As early detection increases the chances of any treatment's success, it is very important, e.g. in case of ovarian cancers early detection is particularly difficult. Survival rate falls from 95% to 35 % in case the cancer is detected at an early stage before metastatic stage or later when it develops into a highly metastatic stage, respectively. Now days, investigators use proteomic-based technology and have developed simple blood tests to diagnose early stage, premetastatic stage in woman with ovarian cancer. These tests are very efficient and they can detect altered protein patterns in the diseased stage.

Prevention: Molecular Basis

Lifestyle plays a significant role in modifying any individual's risk for developing cancer. There are number of evidences which show the adverse effects of obesity and certain behaviors such as smoking, overexposure to UV light, physical inactivity and dietary choices to cancer risk and mortality. These activities can cause alterations in many biological/ metabolic pathways which can in turn lead to cancer initiation and development.

There are various programs which have been initiated to: identify the precise molecular alteration resulting from lifestyle factor; to validate their relevance to cancer initiation or progression; and to develop preventing strategies using chemopreventive agents or alterations in behavior. For example Selenium and Vitamin E Cancer Prevention Trial (SELECT), where, molecular genetics of cancer risk and associations between diet and cancer will be assessed.