THE TERMINOLOGY OF CANCER

Like most scientific disciplines, carcinogenesis has its own language. This section will familiarize you with some of the terms that you will encounter in your study of cancer. Since cancer is a disease that is characterized by uncontrolled or disregulated cell growth, most of the following terms relate to cell growth and differentiation.

Anaplasia. Literally means "without form." Characterized by a marked change from a highly differentiated cell type to one that is less differentiated or more embryonic in nature. Thus, anaplastic tissue is less organized and functional than is the normal tissue. Anaplasia probably occupies the borderline between dysplasia and neoplasia.

Benign. A term applied to neoplasms that are localized and encapsulated. Growth generally occurs via expansion and compression of adjacent tissue. Growth is generally slow, and there may be regression. If the growth is progressive, it is usually orderly and uniform.

Dysplasia. A reversible change in cells, which may include an altered size, shape, and/or organizational relationship. This change usually affects epithelium and often results from chronic irritation.

Hyperplasia. Increased organ or tissue size due to an increase in cell number. Hyperplasia may be physiological (e.g., tissue development and wound healing) or pathological (e.g., nodular liver regeneration in chronic alcoholics). Several hallmarks distinguish neoplasia from hyperplasia.

Neoplasia	**Hyperplasia**
Growth in excess of needs	Not excessive to needs
Purposeless	Purposeful
Persistent	Ceases when stimulus ceases
Irreversible	Reversible
Autonomous	Regulated

Malignant. A term applied to neoplasms that are locally invasive. Growth may be rapid and is disorderly and progressive. Malignant neoplasms often have areas of necrosis. May spread by extension or metastasis.

Metaplasia. A reversible change in which one differentiated cell type is replaced by another cell type.

Metastasis. Presence of a disease process (usually cancer) at a site distant from the site of origin (the primary tumor). Metastasis (v. *metastasize*, adj. *metastatic*) is the primary hallmark of malignancy.

Neoplasia. Literally means "new growth." Often used synonymously with cancer. The pathologist R. A. Willis offered the following definition, " A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissues and which persists in the same excessive manner after cessation of the stimuli which evoked the change."

Tumor. A mass or swelling; one of the cardinal signs of inflammation. By common usage, a tumor is specifically a neoplasm.

CLASSIFICATION OF TUMORS

Neoplasms can be formed in any tissue, and a variety of benign and malignant tumors can occur throughout the body. These tumors are classified using a binomial system based on (1) the tissue or cell type of origin and (2) their actual or predicted behavior (i.e., benign or malignant).

Histogenesis: Tissue Origin

Nearly all tumors arise from either *epithelial* (ectodermal or endodermal) or *mesenchymal* (mesodermal) tissues. Epithelial tissues include lining epithelium (e.g., skin epidermis and the epithelium of the gastrointestinal tract and urinary system) and glands (e.g., pancreas, liver, mammary, prostate, sweat, and sebaceous glands). Mesenchymal or connective tissues include cartilage, bone, muscle, lymphoid,

and hematopoietic cells.

Behavior of Neoplasm

The distinction between benign and malignant tumors is extremely important because the malignancy of a tumor is typically what defines human cancer as a disease state. As noted previously, the ability to metastasize is the definitive characteristic of a malignant neoplasm. Not all malignant tumors metastasize (e.g., CNS tumors, intraocular tumors), but no benign tumors do. Metastasis is the major cause of morbidity and mortality associated with cancer. Treatment is much less likely to be successful once metastasis has occurred. In contrast to malignant tumors, benign neoplasms do not grow beyond their boundaries. However, benign tumors can inflict damage by localized obstruction, compression, interference with metabolism, and even secretion of unneeded hormones. Once a benign tumor is removed, so is its harmful influence. Table 13.1 provides further distinction between benign and malignant neoplasms.

TABLE 13.1 Distinctions between Benign and Malignant Neoplasms

Benign	Malignant
Well differentiated; resembles a cell of origin	Poorly differentiated or anaplastic
Grows by expansion	Grows by expansion and infiltration
Well circumscribed, often encapsulated by a peripheral rim of fibrous tissue	Poorly circumscribed and invades stroma and vessels
Grows at a normal rate	Growth rate usually increased
Few mitotic figures	Frequent mitotic figures
Growth may be limited	Progressive growth
Does not metastasize, seldom dangerous	Metastasis usual; can be fatal
Adequate blood supply	Often outgrows blood supply, becomes necrotic

CARCINOGENESIS BY CHEMICALS

The following definitions should help clarify subsequent discussions about chemical-induced carcinogenesis.

- A *carcinogen*, as defined in this chapter, is a chemical capable of inducing tumors in animals or humans.
- *Carcinogenesis* is the origin or production of cancer; operationally speaking, this includes any tumor, either benign or malignant.
- A *direct-acting* or *primary carcinogen* is a chemical that is reactive enough to elicit carcinogenic effects in the parent, unmetabolized form. Often these chemicals produce tumors at the site of exposure (e.g., alkylating agents, radiation).
- A *procarcinogen* is a chemical that requires metabolism or bioactivation to another chemical form before it can elicit carcinogenic effects (e.g., polycyclic aromatic hydrocarbons).
- A *cocarcinogen* is a chemical that increases the carcinogenic activity of another carcinogen when coadministered with it. While not carcinogenic itself, the agent may act to increase absorption, increase bioactivation, or inhibit detoxification of the carcinogen administered with it.

TABLE 13.3 Classification of Carcinogenic Chemicals

Type	Possible or Probable Mechanism of Action	Examples
Genotoxic carcinogens		
Direct-acting or procarcinogenic	An electrophile, the compound alters the genetic code via mutagenic or clastogenic processes	Bis(chloromethyl) ether, nitrosamines, benzanthracene, epoxides, dimethyl sulfate, nitrosoureas
Inorganic carcinogenic	Alters the fidelity of DNA replication	Cadmium, chromium, nickel
Epigenetic carcinogens		
Solid-state	Mechanical disruption of tissue	Asbestos, metal foils, plastic
Hormonal	Disrupts cellular dedifferentiation, promotes cellular growth	Estrogens, androgens, thyroid hormone, tamoxifen, diethylstilbestrol
Immunosuppressant	Depression of the immune system allows the proliferation of initiated cells or tumors	Azathioprine
Cocarcinogenic	Modifies the response of genotoxic carcinogens when coadministered	Ethanol, solvents, catechol
Promoter	Enhances cell growth, promotes response to initiator or genotoxic carcinogen	Phorbol esters, catechol, ethanol
Cytotoxic	Increases the rate of spontaneous mutation, promotes regenerative cell growth	Trichlorethylene, carbon tetrachloride, chloroform

Early Epidemiologic Evidence

Some of the earliest evidence that exposure to chemicals could play a role in the development of cancer came from the observations of the British physician Sir Percivall Pott. In 1775, Pott reported on a relationship between scrotal cancer and occupation in men who in their youth, had been employed as chimney sweepers. He suggested that the soot to which these men were exposed on the job played a causal role in the development of their cancer. Over the next century, mounting evidence implicated other chemicals and industrial processes in human cancer. In 1884, Bell and Volkman independently reported on the increased prevalence of skin cancer in workers who were exposed to oils that were distilled from coal and shale. Sporadic reports of other occupational exposures and cancer began appearing in the literature. In 1895, Rehn reported on bladder cancer in aniline dye workers in Germany.

In the early 1900s, radiation was associated with lung tumors in uranium miners and in skin tumors and leukemia in technicians working with the recently discovered X rays.

If it were true that exposure to chemicals could cause cancer in humans, researchers at the beginning of the twentieth century were hopeful that they could be identified and their mechanisms of action studied. Unfortunately, and a bit ironically, early attempts at reproducing cancer in laboratory animals were fruitless. Experimental validation of Pott's original hypothesis finally came in 1915, when the Japanese pathologists Yamagiwa and Ichikawa reported that rabbits developed malignant skin tumors following repeated topical applications of coal tar. The line of research that Pott began was brought full circle when, in the 1930s, a group of investigators led by Cook and Kennaway implicated polycyclic aromatic hydrocarbons as putative carcinogens in coal tar and other industrial oils. They identified benzo[a]pyrene as the first carcinogenic hydrocarbon of known structure isolated from coal tar. Soon afterward, the structures of other chemical carcinogens were identified and their carcinogenic effects replicated in experimental animal models.

The Somatic Mutation Theory

In the early 1900s very little was known about the mechanism of cancer induction by chemicals. Theodor Boveri is often credited with the proposition that cancer involved a permanent alteration of the genetic material in somatic cells. In what became known as the *somatic mutation theory*, he attributed cancer to an " abnormal chromatin complex, no matter how it arises. Every process which

brings about this chromatin condition would lead to a malignant tumor." It is important to point out, however, that this theory had as its basis only gross morphological observations of cancer cells. It was only after the pioneering work of Watson and Crick in 1950s, that it became evident that interference with DNA basepairing could be a mechanism by which chemicals induced mutation.
A great deal of evidence has now accumulated in support of the somatic mutation theory (i.e., a genetic mechanism of cancer). Since the early 1970s many carcinogens have been shown to produce permanent, heritable changes in DNA. It has further been shown that these changes are involved in the carcinogenic process. Smart (1994) has described several lines of evidence that support a genetic mechanism for cancer.

- Cancer is a heritable change at the cellular level.
- Tumors are generally clonal in nature.
- Many carcinogens or their activation products can form covalent bonds with DNA and produce mutations.
- The inheritance of certain recessive mutations in genes associated with genomic integrity predisposes affected individuals to cancer.
- Most cancers display chromosomal abnormalities.
- The phenotypic characteristics of a tumor cell can be transferred to a nontumor cell by DNA transfection.

Initiation and Promotion

Since the eighteenth century, investigators have realized that the carcinogenic process involved a peri=od of latency following exposure to chemical carcinogens prior to the appearance of any clinical symptoms. Early attempts at inducing cancer with chemicals in experimental animals met with little success. It was not until the work of Yamigawa and Ichikawa that it was realized that the generation of cancer in experimental animals required long-term repeated exposures to chemicals. With this discovery, researchers quickly developed animal models with which to test the carcinogenic potency of chemicals and mixtures. The most common was the mouse skin model, in which repeated applications of a potential carcinogen were applied to the shaved back of a mouse and the number of tumors generated and the time required for their development was recorded. In the early 1940s,

researchers working with rodent skin models discovered that croton oil could stimulate the rapid development of tumors but only if it was applied after treatment with polycyclic aromatic hydrocarbons. Rous and coworkers were the first to use the terms *initiation* and *promotion* to describe two stages of carcinogenesis observed in the experimental induction of skin tumors in rodents. The term *progression* was added later to describe the sequence of events leading to the development of malignant tumors. These three stages are remarkably similar to those described in the development of human skin cancer, induced by soot and paraffin oils.
It is now known that initiation, the first step in this process, involves an irreversible mutation in the DNA of a somatic cell. Chemical initiators are usually electrophiles or metabolically activated to electrophiles . These chemicals bind to nucleophilic centers in DNA, forming DNA adducts. If the DNA is replicated prior to repair of an adduct, a mutation can be " fixed" in the DNA of the daughter cell. This mutation essentially primes
the cell for later steps in neoplastic development. Most initiators are mutagens and are thus classified as *genotoxic* carcinogens.
Chemicals that act as tumor promoters may not, by themselves, be carcinogenic. However, if given subsequent to an initiating agent, they increase either the number of tumors or decrease the latency period or both. Tumor promoters typically do not bind DNA; rather, they allow for the clonal expansion of initiated cells by providing a selective growth advantage. For this reason, promoters are considered *epigenetic* (or *nongenotoxic*) carcinogens. For example, the active ingredients of the first tumor promoter, croton oil, are phorbol esters. These compounds mimic endogenous molecules that trigger cell proliferation, thus allowing initiated cells to proliferate. Progression, the third stage of the experimental carcinogenic process, is less well characterized. In general, progression is thought to involve the accumulation of further genetic alterations in a population of initiated cells that have been provided a growth advantage through promotion. These changes ultimately lead to a malignant tumor
.
A concept that is important in the context of neoplastic progression is that of tumor cell heterogeneity. Investigators studying leukemias and lymphomas have demonstrated that these cancers are almost universally clonal in origin. While there is also evidence of this type of clonal origin in solid tumors (i.e., carcinomas and sarcomas), by the time clinical signs of cancer are evident, the cells that

make up tumor have usually developed a certain amount of genotypic and phenotypic diversity. Many researchers believe that the cellular heterogeneity observed in these tumors is the result of genetic instability acquired during tumor progression. Genetic instability suggests that the DNA in tumor cells is mutated at higher rates than the surrounding normal cells rapidly producing subclones. Some of these clones would have adaptations, such as the ability to escape the host's defense mechanisms or invade surrounding tissue that give them a selective advantage. These clones eventually grow to dominate the tumor population. Multiple rounds of this type of selection lead to populations of cells that are increasingly abnormal on a genotypic and phenotypic level and as a result, more aggressive and invasive.

While the initiation-promotion model was first described in a rodent skin model, the process has been experimentally reproduced in other organs such as liver, colon, lung, prostate, and mammary gland. Our experience with both initiators and promoters indicates that many are organ specific. There are, however several features of the initiation–promotion model that remain relatively constant .

- Exposure to a sub-threshold dose of an initiator alone results in few, if any tumors.
- Exposure to a sub-threshold dose of an initiator followed by repeated exposure to a promoter results in many tumors.
- Exposure to a promoter will produce tumors even if there has been a latent period following exposure to an initiator. Thus, initiation is irreversible.
- In contrast, if initiation is not followed by promotion of sufficient duration, no tumors are produced. Thus, the effects of tumor promoters are reversible in the early stages

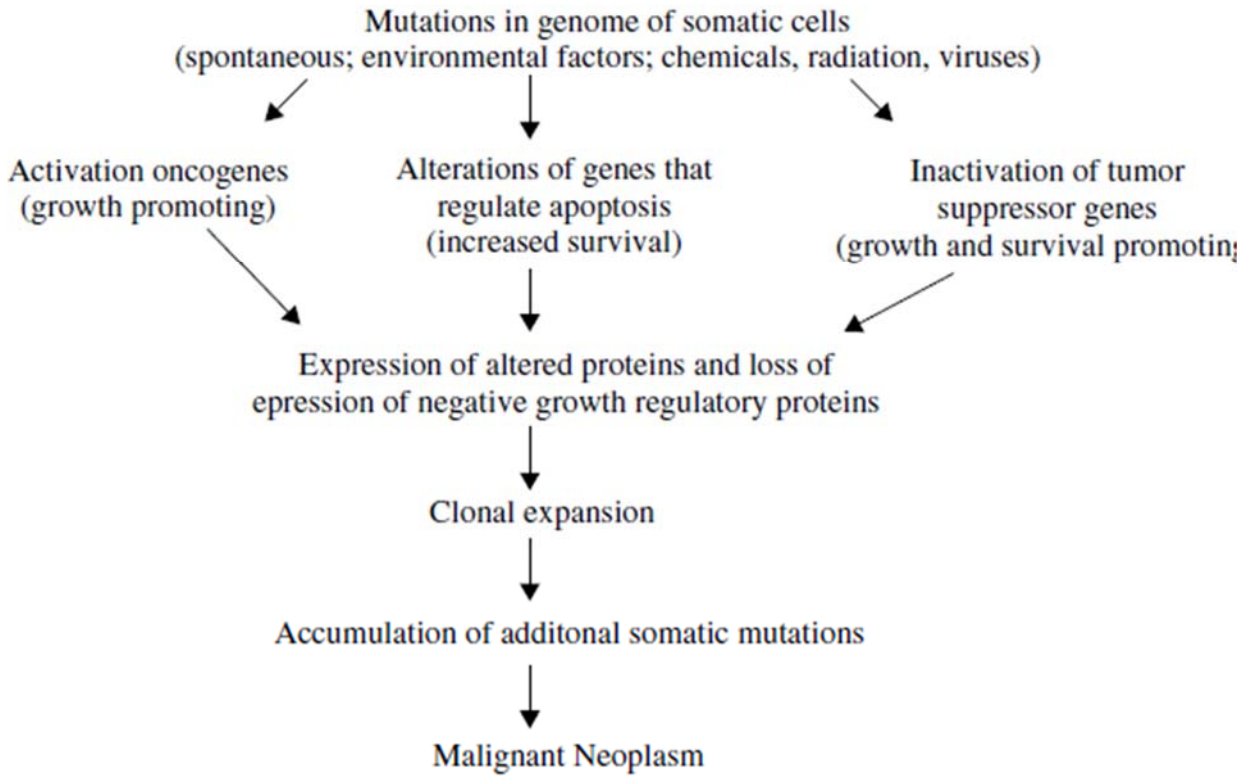
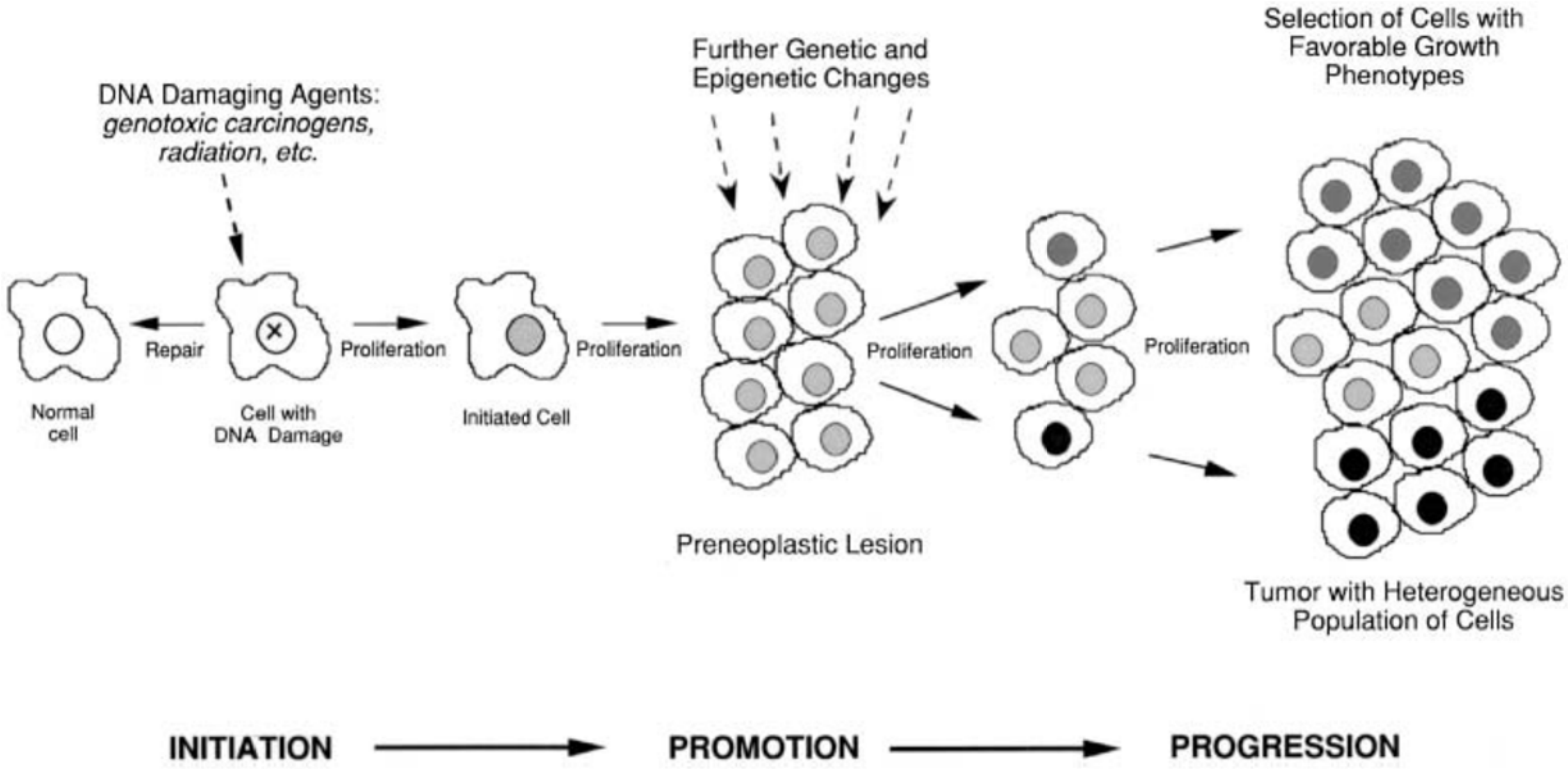


Figure 12.3 General overview of the cancer process.


DNA Damaging Agents:
genotoxic carcinogens,
radiation, etc.
Further Genetic and
Epigenetic Changes
Selection of Cells with
Favorable Growth
Phenotypes
Repair
Proliferation
Proliferation
Proliferation
Proliferation
Normal
cell
Cell with
DNA Damage
Initiated Cell
Preneoplastic Lesion
Tumor with Heterogeneous
Population of Cells
INITIATION
PROMOTION
PROGRESSION

Electrophilic Theory

The nature of the initiation step in chemical carcinogenesis was the subject of much scientific inquiry and debate for decades. Until 1940, the only known chemical carcinogens were aromatic hydrocarbons and amines. Soon afterward, other aliphatic chemicals were also shown to be carcinogenic and by the 1960s the various chemical carcinogens belonged to over a dozen chemical classes .
Attempting to explain this structural diversity, in 1969 James and Elizabeth Miller hypothesized that "most, if not all, chemical carcinogens either are, or are converted to, reactive electrophilic derivatives which combine with nucleophilic growth crucial tissue components, such as nucleic acids or proteins."
In what became known as the *electrophilic theory of chemical carcinogenesis*,

In the 1950s **Elizabeth and James Miller** observed that a diverse array of chemicals could produce cancer in rodents. In an attempt to explain this, they hypothesized that many carcinogens are metabolically activated to electrophilic metabolites that are capable of interacting with nucleophilic sites in DNA. The Millers termed this the electrophilic theory of chemical carcinogenesis. From this concept of metabolic activation, the important terms parent, proximate, and ultimate carcinogen were developed. A parent carcinogen is a compound that must be metabolized in order to have carcinogenic activity; a proximate carcinogen is an intermediate metabolite requiring further metabolism and resulting in the ultimate carcinogen, which is the actual metabolite that covalently binds to the DNA. The cell has many defense systems to detoxify the carcinogenic species, including cellular antioxidants and nucleophiles as well as a whole host of phase I and phase II enzymes. In addition reactive carcinogenic species may bind to non critical sites in the cell, resulting in detoxification, or they can undergo spontaneous decomposition. If the carcinogenic species binds to DNA, the adducted DNA can be repaired and produce a normal cell. If there is error in the repair of the DNA or the DNA adduct is not repaired before the cell replicates, an error in the newly synthesized DNA could occur, and if so, a mutation would occur in the DNA of the daughter cell. If this change has occurred in a critical gene, for example, in a protooncogene or tumor suppressor gene, it would represent an important mutagenic event(s) in carcinogenesis. The mutationally altered cell or "initiated cell" has an altered genotype and may remain dormant (not undergo clonal expansion) for the lifetime of the animal. However, additional mutations or "hits" in critical genes followed by clonal expansion could lead to tumor development. In addition to this mechanism, chemical carcinogenesis in experimental models can be divided into at least three stages: termed initiation, promotion, and progression; this model is thus often referred to as the initiation/promotion model of chemical carcinogenesis. As mentioned above, the "initiated cell" may remain dormant (not undergo clonal expansion) for the lifetime of the animal. However, if the animal is repeatedly exposed to a tumor promoter, it will provide a selective growth advantage to the "initiated cell," which will clonally expand and eventually produce a benign tumor. This process is termed tumor promotion and is an epigenetic process favoring the growth of cells with an altered genotype. The development of a malignant tumor from a benign tumor encompasses a third step, termed progression and involves additional genetic changes.

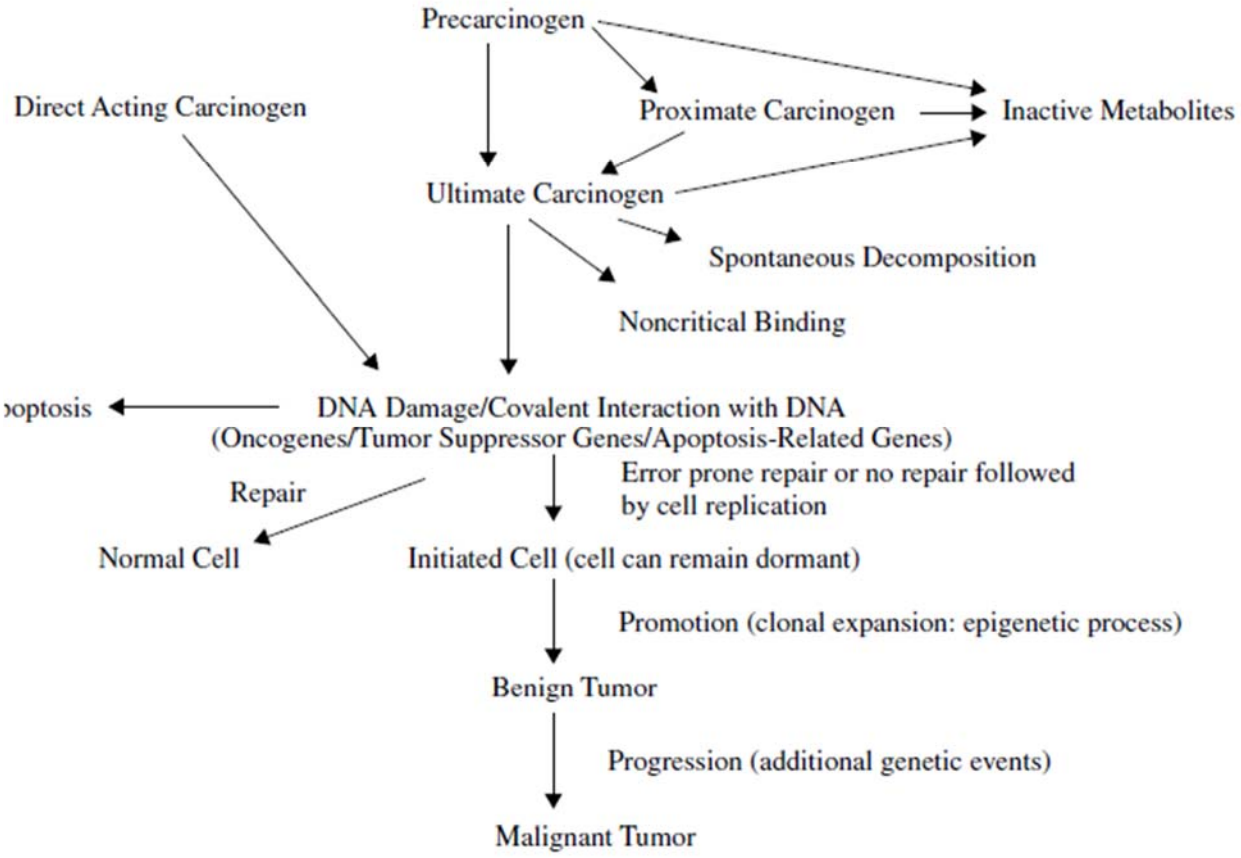


Figure 12.10 General aspects of chemically induced carcinogenesis.

METABOLIC ACTIVATION OF CHEMICAL CARCINOGENS AND DNA ADDUCT FORMATION

Having described the general aspects of chemical carcinogenesis including the initiation-promotion model, we now examine some aspects of chemical carcinogenesis in more detail. Metabolic activation of chemical carcinogens by cytochromes P450 is well documented. The metabolism of benzo[a]pyrene has been extensively studied and at least 15 major phase I metabolites have been identified. Many of these metabolites are further metabolized by phase II enzymes to produce numerous different metabolites. Extensive research has elucidated which of these metabolites and pathways are important in the carcinogenic process. As shown in Figure 12.12, benzo[a]pyrene is metabolized by cytochrome P450 to benzo[a]pyrene-7,8 epoxide, which is then hydrated by epoxide hydrolase to form benzo[a]pyrene-7,8-diol. Benzo[a]pyrene-7,8-diol is considered the proximate carcinogen since it must be further metabolized by cytochrome P450 to form the ultimate carcinogen, the bay region diol epoxide, (+)-benzo[a]pyrene-7,8-diol-9,10-epoxide-2. It is this reactive intermediate that binds covalently to DNA, forming DNA adducts. (+)-Benzo[a]pyrene-7,8-diol-9,10-epoxide-2 binds preferentially

to deoxyguanine residues, forming *N*-2 adduct. (+)-Benzo[a]pyrene-7,8-diol-9,10-epoxide-2 is highly mutagenic in eukaryotic and prokaryotic cells and carcinogenic in rodents. It is important to note that not only is the chemical configuration of the metabolites of many polycyclic aromatic hydrocarbons important for their carcinogenic activity, but so is their chemical conformation/stereospecificity (Figure 12.12). For example, four different stereoisomers of benzo[a]pyrene-7,8-diol-9,10 epoxide are formed. Each one only differs with respect to whether the epoxide or hydroxyl groups are above or below the plane of the flat benzo[a]pyrene molecule, but only one, (+)-benzo[a]pyrene-7,8-diol-9,10-epoxide-2, has significant carcinogenic potential. Many polycyclic aromatic hydrocarbons are metabolized to bay-region diol epoxides. The bay-region theory suggests that the bay-region diol epoxides are the ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons.

DNA can be altered by strand breakage, oxidative damage, large bulky adducts, and alkylation. Carcinogens such as *N*-methyl-*N* _-nitro-*N*-nitrosoguanidine and methyl methanesulfonate alkylate DNA to produce *N*-alkylated and *O*-alkylated purines and pyrimidines. Ionizing radiation and reactive oxygen species commonly oxidize guanine to produce 8-oxoguanine. Formation of DNA adducts may involve any of the bases, although the *N*-7 position of guanine is one the most nucleophilic sites in DNA. Of importance is how long the adduct is retained in the DNA. (+)-Benzo[a]pyrene-7,8-diol-9,10-epoxide-2 forms adducts mainly at guanine *N*-2, while aflatoxin B1 epoxide, another well-studied rodent and human carcinogen, binds preferentially to the *N*-7 position of guanine. For some carcinogens there is a strong correlation between the formation of very specific DNA-adducts and tumorigenicity. Quantitation and identification of specific carcinogen adducts may be useful as biomarkers of exposure.

Importantly, the identification of specific DNA-adducts has allowed for the prediction of specific point mutations that would likely occur in the daughter cell provided that there was no repair of the DNA-adduct in the parent cell. As will be discussed in a later section, some of these expected mutations have been identified in specific oncogenes and tumor suppressor genes in chemically induced rodent tumors, providing support that the covalent carcinogen binding produced the observed mutation. In several cases, specific base pair changes in p53 tumor suppressor gene in human tumors are associated with a mutational spectrum that is consistent with exposure of the individual to a specific carcinogen. For example, the mutation spectra identified in p53 in human tumors thought to result from the exposure of the individual to ultraviolet radiation (UVR), aflatoxin, and benzo[a]pyrene (from cigarette smoke), are consistent with the observed specific mutational damage in p53 induced by these agents in experimental cellular systems.

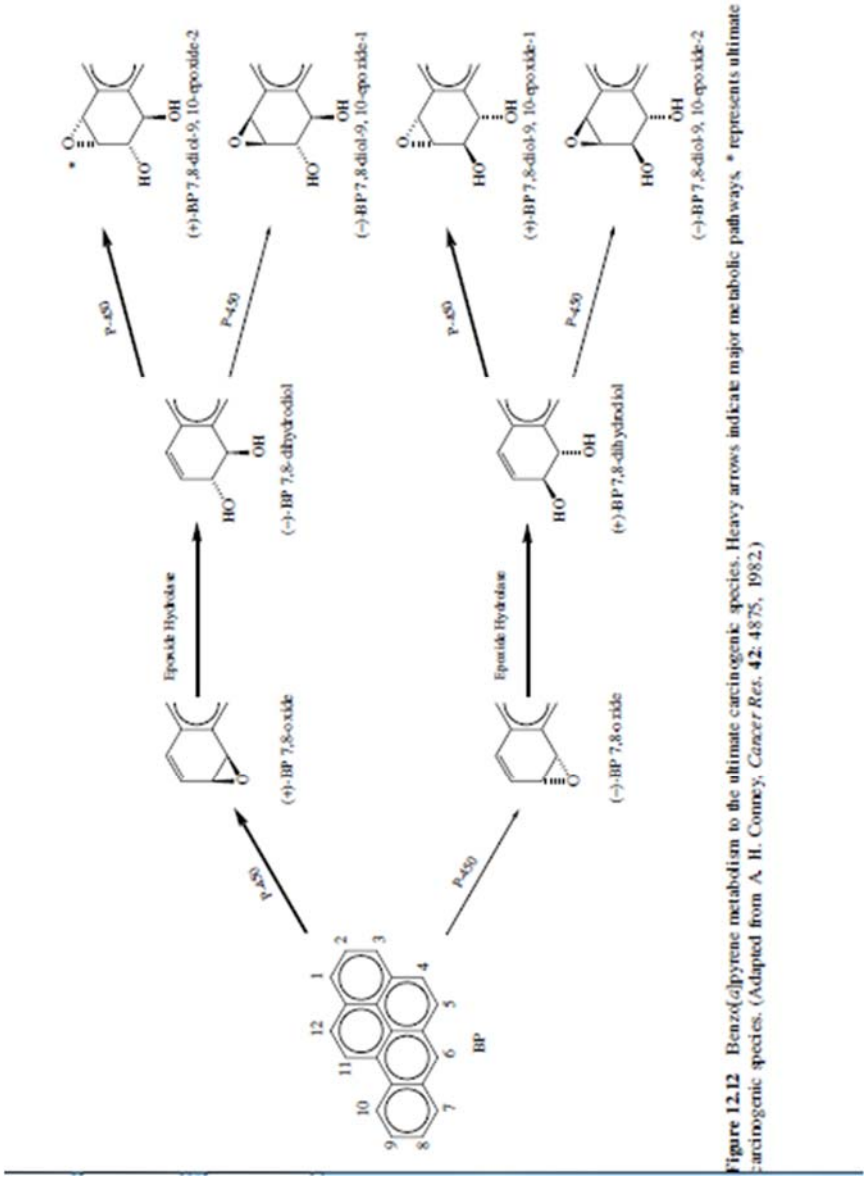


Figure 12.12 Benz[*a*]pyrene metabolism to the ultimate carcinogenic species. Heavy arrows indicate major metabolic pathways. * represents ultimate carcinogenic species. (Adapted from A. H. Conney, *Cancer Res.* **42**: 4875, 1982.)

Carcinogenicity and Mutagenicity

The relationship between mutagenicity and carcinogenicity as it related to the effects of ionizing radiation has been known since the early part of the twentieth century. Sufficient doses of ionizing radiation produce large structural alterations in the genetic material. The first chemical mutagens to be identified were the nitrogen and sulfur mustards (mustard gas). These chemicals were studied because their biological effects were similar to those of ionizing radiation. The nitrogen mustards and other *radiomimetic* compounds have the ability to form crosslinks between strands of DNA or between DNA and proteins. When such lesions are not repaired, large-scale alterations in the DNA can occur. These types of effects are referred to as *clastogenic*, and chemicals that cause them are known as clastogens. The identification of DNA as the genetic material along with the proposition that many chemical carcinogens are, or become, metabolically activated to reactive electrophiles paved the way for the identification of a strong link between chemical mutagenicity and carcinogenicity

Genotoxic and Epigenetic Carcinogens

With the realization that carcinogens elicited their effects via diverse mechanisms, a number of

classification schemes based on carcinogenic mechanism were developed. One popular scheme was offered by Williams (Table 13.3). This scheme divides chemical carcinogens into two broad categories based on whether they act in a genotoxic fashion. Accordingly, the two main groups of carcinogenic chemicals are

- Genotoxic carcinogens
- Epigenetic (or nongenotoxic) carcinogens1

Genotoxic carcinogens are those chemicals that are capable of modifying the primary sequence of DNA (i.e., initiators). This group includes chemicals that induce mutational and clastogenic changes or changes in the fidelity of DNA replication. Epigenetic carcinogens do not alter the primary sequence of DNA; instead they can affect cell proliferation and differentiation by a number of mechanisms including cytotoxicity and compensatory cell proliferation, receptor mediated events, and by altering the expression or repression of certain genes and cellular events related to cell proliferation and differentiation. It is estimated that at least 40 percent of carcinogens identified by rodent bioassays elicit their affects via an epigenetic mechanism. Many epigenetic agents favor the proliferation of cells with altered genotypes due to an interaction with an initiating carcinogen. While epigenetic carcinogens and tumor promoters share many of the same characteristics, there is some debate regarding whether classic tumor promoters should be considered carcinogens at all. However, since tumor promoters can affect the proliferation of cells that have spontaneous as well as chemically induced mutations, we will group them with the epigenetic carcinogens.

The mechanism of a chemical's carcinogenic action affects the manner in which it is treated for regulatory purposes. Based on early theories, it was assumed that even a single molecule of a genotoxic chemical could irreversibly damage DNA and that each additional exposure could add to the damage from previous exposures. Thus, regulators initially assumed that there is no safe level of exposure or " threshold" below which harmful effects do not occur. Now, however, many carcinogens, particularly epigenetic carcinogens are thought to elicit their effects in a manner consistent with a threshold.

Epigenetic Mechanisms

Although the precise mechanisms of carcinogenesis by epigenetic chemicals are unknown, progress is being made toward understanding the organ- and species-specific effects of certain classes of epigenetic carcinogens. Some effects of these chemicals appear to be mediated by cytotoxic insult and a compensatory regenerative response while others have been shown to act via receptor-mediated events resulting in the altered transcription of critical cellular genes. Alteration of DNA methylation status such that critical genes are expressed or are inactivated inappropriately is another epigenetic mechanism that is currently receiving increased attention, although as yet, it is uncertain how chemicals might affect this process.

Some chemicals that are not directly genotoxic, but are carcinogenic in chronic rodent bioassays have been shown to exhibit cytotoxic properties. It has been shown that many of these chemicals produce necrosis or cell death due to cytotoxicity at the target organ. This is usually followed by regenerative cell proliferation. The organ- or cell-type-specific effects of carcinogens that induce cytotoxicity may be due to the high concentration of the chemical at that organ or the selective toxicity directed at specific cell populations. Cells in the target tissue could become initiated following an insult with a cytotoxic chemical due to (1) spontaneous mutations produced by defective mitosis or inefficient repair occurring during multiple rounds of DNA replication during regenerative cell proliferation or (2) generation of DNA damage by oxygen free radicals, produced by lipid peroxidation or by recruited inflammatory cells. Such initiated cells would then have a selective growth advantage due to the production of various stimuli (e.g., growth factors) produced by proliferating cells. Examples of chemicals that may work via these types of mechanisms include chloroform, carbon tetrachloride, and saccharine.

There is an increasing amount of evidence that suggests that many epigenetic carcinogens activate receptors and as a result, elicit changes in the expression of critical target genes involved in cellular functions ranging from signal transduction, cell proliferation, and differentiation to apoptosis and cell-to-cell communication. Some of these chemicals and their receptors are shown in Table 13.4. The changes in gene expression induced by certain epigenetic carcinogens may mimic the effects of endogenous growth factors and hormones that similarly affect these cell functions. The effects of natural hormones, growth factors, and dietary constituents on promotion of neoplasia suggest that endogenous tumor promotion and epigenetic carcinogens have common links. The tissue-specific effects of some chemicals may be due to the predominance of particular signaling pathways in a given cell or tissue type. Because of the increased understanding of nongenotoxic mechanisms of action of epigenetic carcinogens, endpoints such as proliferation, differentiation, apoptosis, cell-to-cell communication, and the induction of gene expression have all been used in the assessment of epigenetic

carcinogens. Experimental evidence that some of these compounds elicit their effects via receptors provides a solid basis for the contention that there exists a threshold level under which epigenetic carcinogens would not exert carcinogenic activity.

Another epigenetic mechanism that received more attention in the late 1990s is the alteration of DNA methylation patterns in neoplastic cells. Most cells in the body contain the same genetic information. Yet somehow, different cell types express only a subset of that genetic code that is required for the cell to function properly. Cell differentiation is almost always achieved without altering the primary sequence of DNA, yet the phenotypic characteristics of the cell are usually stable and can be passed on to daughter cells during cell division. Much of the control of the gene expression that ultimately determines cell phenotype is maintained by the addition of methyl groups to the 5^2 carbon of cytosine residues in cellular DNA, particularly at CpG dinucleotide sequences. The promoter and enhancer elements of many genes have regions high in CpG dinucleotides. There is an inverse correlation between gene expression levels and the degree of methylation in these regions. That is, actively transcribed genes have low levels of methylation (hypomethylation) in their promoter regions while transcriptionally silent genes have heavily methylated (hypermethylation) promoter regions. It has been hypothesized that changes in methylation status play an important role in the neoplastic progression of tumor cells. Once in place, changes in methylation status could be passed to daughter as permanent epigenetic changes. There is evidence for this type of mechanism in the inactivation of the p16 tumor suppressor gene in human tumors by hypermethylation of the promoter region. It is still unclear how epigenetic carcinogens might affect this mechanism of gene regulation, but it has become increasingly apparent that alteration of DNA methylation patterns do play a role in the progression of some tumors.

TABLE 13.4 Some Epigenetic Carcinogens and the Receptors They Activate

Chemical	Receptor
Tetrachloro dibenzo-*p*-dioxin (TCDD)	Ah receptor
12-*o*-Tetradecanoylphorbol-13-acetate (TPA)	Protein kinase C
Peroxisome proliferating compounds	Peroxisome proliferator-activated receptor (PPAR)
Estrogenic compounds	Estrogen receptor
Okadaic acid	Protein phosphatase-2A

MOLECULAR ASPECTS OF CARCINOGENESIS

Oncogenes

Thus far, our study of carcinogenesis has focused on information gathered by researchers studying the induction of cancer by chemicals. There was however, another group of cancer researchers who believed that infectious agents, namely viruses were actually the cause of cancer. While we now know that this is not the case for the overwhelming majority of human cancer, the work of these investigators has provided us with some of the most important information on the molecular mechanisms of cancer. Peyton Rous and his coworkers discovered the first known tumor virus in 1909. They demonstrated that virus particles extracted from a sarcoma in one chicken could produce similar tumors when injected into other chickens. The Rous sarcoma virus as it became known was eventually shown to be an *oncogenic* (from the Greek word *onkos*—mass or swelling) retrovirus. In the decades that followed, many other oncogenic retroviruses were discovered. These viruses were active in other avian species, rodents, and even primates.

Retroviruses encode their genetic material in RNA rather than DNA like other organisms. The genome of the retrovirus is limited to several genes that are critical to the production of more virus particles. Following infection, the viral RNA is transcribed into DNA by the enzyme reverse transcriptase. The newly created double stranded DNA integrates into the DNA of the host cell, where the strong promoter sequences of the virus induce the host cell's nuclear machinery to express the viral genes and produce new viral particles. Some oncogenic retroviruses, such as the Rous sarcoma virus, have the ability to rapidly " transform" normal cells into cancer cells. Researchers working with these so called *acute transforming retroviruses* hypothesized that they contained a one or more genes responsible for their rapid transforming ability.

Eventually, a gene responsible for the transforming ability of the Rous sarcoma virus was

discovered. It was named *src* for sarcoma, and it was the first *oncogene* or gene capable of inducing cancer identified. In the years that followed, oncogenes from other acute transforming retroviruses were identified, each of these genes was named with a three-letter identifier that corresponded to the virus from which they were first isolated: *ras* from the rat sarcoma virus, *myc* from the avian myelocytomatosis virus, *sis* from the simian sarcoma virus, and *fes* from the feline sarcoma virus. It was ultimately demonstrated that the oncogenes responsible for the transforming ability of the oncogenic retroviruses had normal, highly conserved counterparts in the cells of a wide variety of prokaryotic and eukaryotic organisms. It stood to reason that if these genes had been conserved over so many years of evolutionary history, they must play an absolutely critical role within the cell. These normal cellular genes appear to have been transduced or in essence " stolen" by retroviruses. Following transduction, the cellular genes became " activated," that is, altered in ways that made them oncogenic. The method of activation was not the same for each oncogene. Some like *erb* B were activated by a deletion of several hundred basepairs of DNA from the coding region of the gene, some like *myc* were activated by gene amplification, while others like *ras* were activated by a single-point mutation. The normal cellular counterparts to the oncogenes of the transforming retroviruses were called *protooncogenes.* Subsequently protooncogenes were shown to be activated in a number of human tumors. Many lines of experimental evidence converged when it was shown that oncogenes that had been activated by mutation with a carcinogenic chemical could transform normal cells into cancer cells. We now know that the same oncogenes are often activated in tumors of the same cell type, whether the tumors arose spontaneously or were virally or chemically induced.
A word about nomenclature should be mentioned here. The student may encounter several notations associated with the names of oncogenes (*oncs*). The notation *v-onc* (e.g., *v-sis*) is used to distinguish an oncogene of viral origin from a similar oncogene of cellular origin (*c-onc*, e.g., *c-sis*). It should also be noted that not all oncogenes have been discovered in transforming retroviruses.

protooncogenes that commonly occur in human tumors include point mutation, gene rearrangement, gene amplification, chromosomal translocation, and increased transcription.

TABLE 13.6 Oncogenes Activated in Human Tumors

Oncogene	Neoplasm(s)	Lesion
abl	Chronic myelogenous leukemia	Translocation
erbB-1	Squamous cell carcinoma; astrocytoma	Amplification
erbB-2 (neu)	Adenocarcinoma of the breast, ovary, and stomach	Amplification
gip	Adenocarcinoma of the ovary and adrenal gland	Point mutations
gsp	Thyroid carcinoma	Point mutations
myc	Burkitt's lymphoma	Translocation
	Carcinoma of lung, breast, and cervix	Amplification
L-*myc*	Carcinoma of lung	Amplification
N-*myc*	Neuroblastoma, small cell carcinoma of lung	Amplification
H-*ras*	Carcinoma of colon, lung, and pancreas; melanoma	Point mutations
K-*ras*	Acute myelogenous and lymphoblastic leukemia; thyroid carcinoma, melanoma	Point mutations
N-*ras*	Carcinoma of the genitourinary tract and thyroid; melanoma	Point mutations
ret	Thyroid carcinoma	Rearrangement
K-*sam*	Carcinoma of stomach	Amplification
trk	Thyroid carcinoma	Rearrangement

Tumor Suppressor Genes
Demonstration of the existence of cellular oncogenes and knowledge of their function as positive regulators of cell growth provided an obvious mechanism by which chemicals could induce the carcinogenic process. The thinking was that an activated oncogene could force the cell and its

descendants into unneeded rounds of division ultimately resulting in a tumor. However, there was a problem with such a simplistic view. Researchers soon demonstrated that when tumor cells were fused with normal cells, the resulting hybrid cells were usually nontumorigenic. Thus the transforming ability of oncogenes could be reversed or controlled by some other factor produced by normal cells. It was eventually discovered that normal cells carried genes that coded for proteins that function as negative regulators of cell growth. These genes came to be called *tumor suppressor genes*. There now exists much evidence supporting the existence of tumor suppressor genes and their functions as negative regulators of cell growth. To date, approximately 20 putative tumor suppressor genes have been identified, although, for many of these, a function is not well understood. Like the oncogenes, the products of tumor suppressor genes appear to have diverse functions within the cell. These functions include cell cycle control, transcriptional regulation, regulation of signal transduction, maintenance of cellular structure, and DNA repair.

TABLE 13.7 Tumor Suppressor Genes in Human Cancer and Genetic Disease

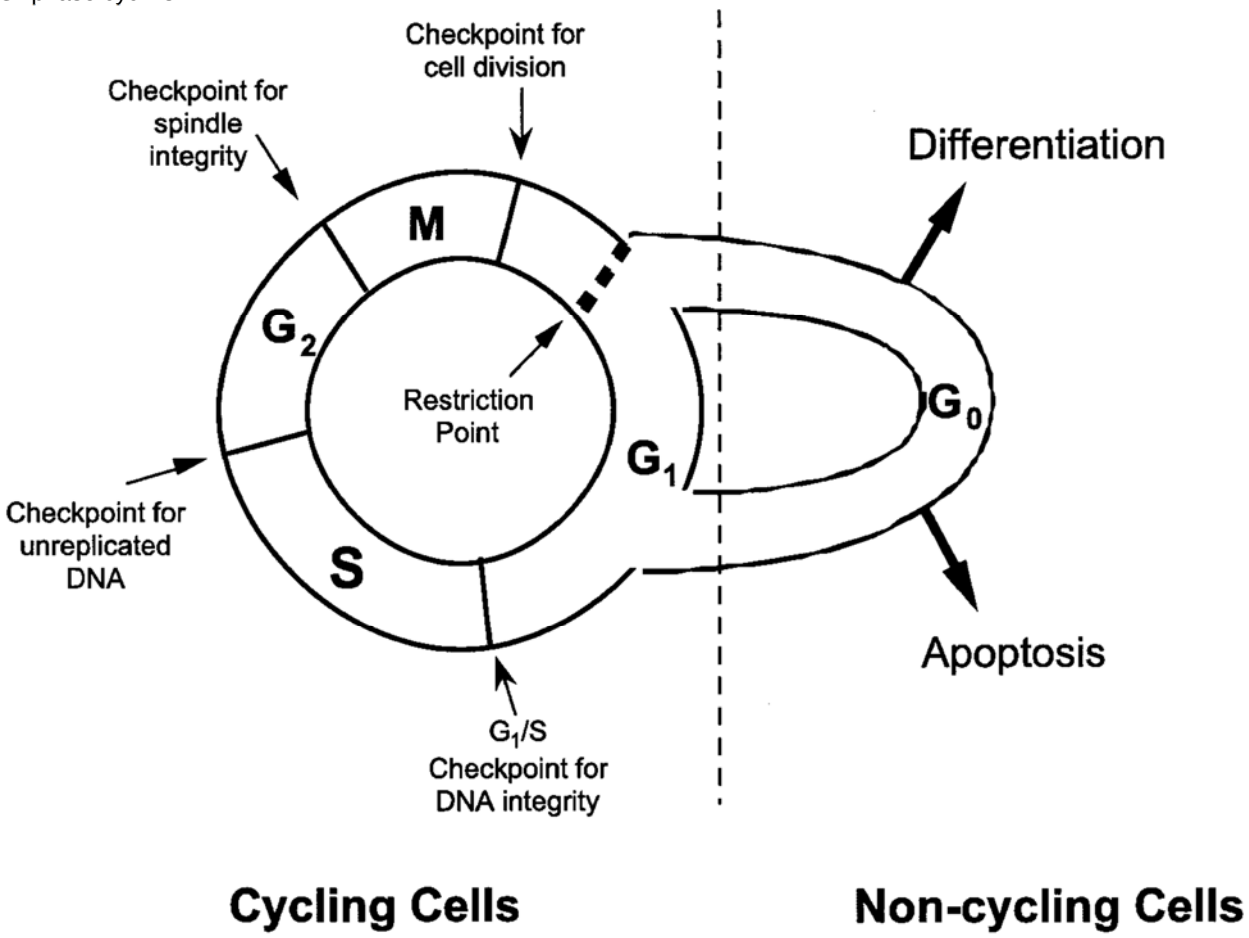
Gene	Consequence of loss	Function of encoded protein
Rb	Retinoblastoma and osteosarcoma	Binds and sequesters the transcription factor E2F to maintain cells in G_0 of cell cycle
p53	Li-Fraumeni syndrome inactivated in >50% of human cancers	Transcription factor with multiple functions, including cell cycle progression, detection of DNA damage, and apoptosis
p16	Familial melanoma, pancreatic cancer	Inhibits CDK4 to block cell cycle progression
Wt1	Wilms' tumor/nephroblastoma	Transcription factor required for renal development
VHL	Von Hippel–Lindau syndrome renal cell carcinoma	Negative regulation of hypoxia-inducible mRNAs
NF1	Neurofibromatosis type 1 schwannoma and glioma	GTPase-activating protein (GAP), which regulates signaling through *ras*
NF2	Neurofibromatosis type 2 acoustic nerve tumors and meningiomas	Connects cell membrane proteins with the cytoskeleton
BRCA1	Familial and sporadic breast and ovarian cancer, also prostate and colon cancers	Secreted growth factor
BRCA2	Breast cancer (female and male) also prostate cancer	Unknown function
DCC	Colon cancer	Cell adhesion molecule
APC	Familial and sporadic adenomatous polyposis colorectal tumors	Interacts with catenins, proteins involved in signaling pathway for tissue differentiation
MMR	Hereditary nonpolyposis colorectal cancer	Mediates DNA mismatch repair

The Cell Cycle and Apoptosis

It is important to discuss some of the processes that govern the life and death of cells to better understand how oncogenes and tumor suppressor genes are involved in these processes. As pointed out previously, proto-oncogenes function in various capacities in the transduction of signals for cell growth and differentiation within and between cells. In normal cells, replication of the DNA and cell division is stimulated by the presence of growth factors that bind receptors at the cytoplasmic membrane and initiate a cascade of intracellular signals. Once these signals reach the nucleus they cause the transcription of a complex array of genes, producing proteins that mediate progression of the cell through the cell cycle culminating in mitosis or cell division.

The cell cycle is divided into five phases. The length of each of these phases can vary depending on factors such as cell type and localized conditions within the tissue. After completing mitosis (M), daughter cells enter the Gap 1 (G_1) phase. If conditions are favorable, cells enter the synthesis (S) phase of the cycle, where the entire genome of the cell is replicated during DNA synthesis. Following S phase, cells enter the Gap 2 (G_2) phase before proceeding through mitosis again. There is a critical boundary early in G_1 called the *restriction point.* This is the point at which the cell must make a decision to (1) enter the cell cycle again or (2) move into a state of quiescence also known as G_0 phase. Once in G_0 phase, the cell can either remain in a state of replicative quiescence until it receives a signal to divide again or it can proceed down a path that leads either to terminal differentiation or to apoptosis.

Movement of the cell through the cell cycle is controlled by an enormously complex network of proteins many of which are expressed in a phase-specific fashion. Several major groups of these proteins have been studied to date. These include cyclins, cyclin-dependent kinases (CDKs), cyclinactivating kinases (CAKs), and CDK inhibitory proteins. The cyclins and CDK proteins are categorized by the stage of the cell cycle in which they are the primarily active. The binding of the appropriate growth factor at the cell surface starts a signaling cascade that ultimately leads to the expression of the G_1 phase cyclins

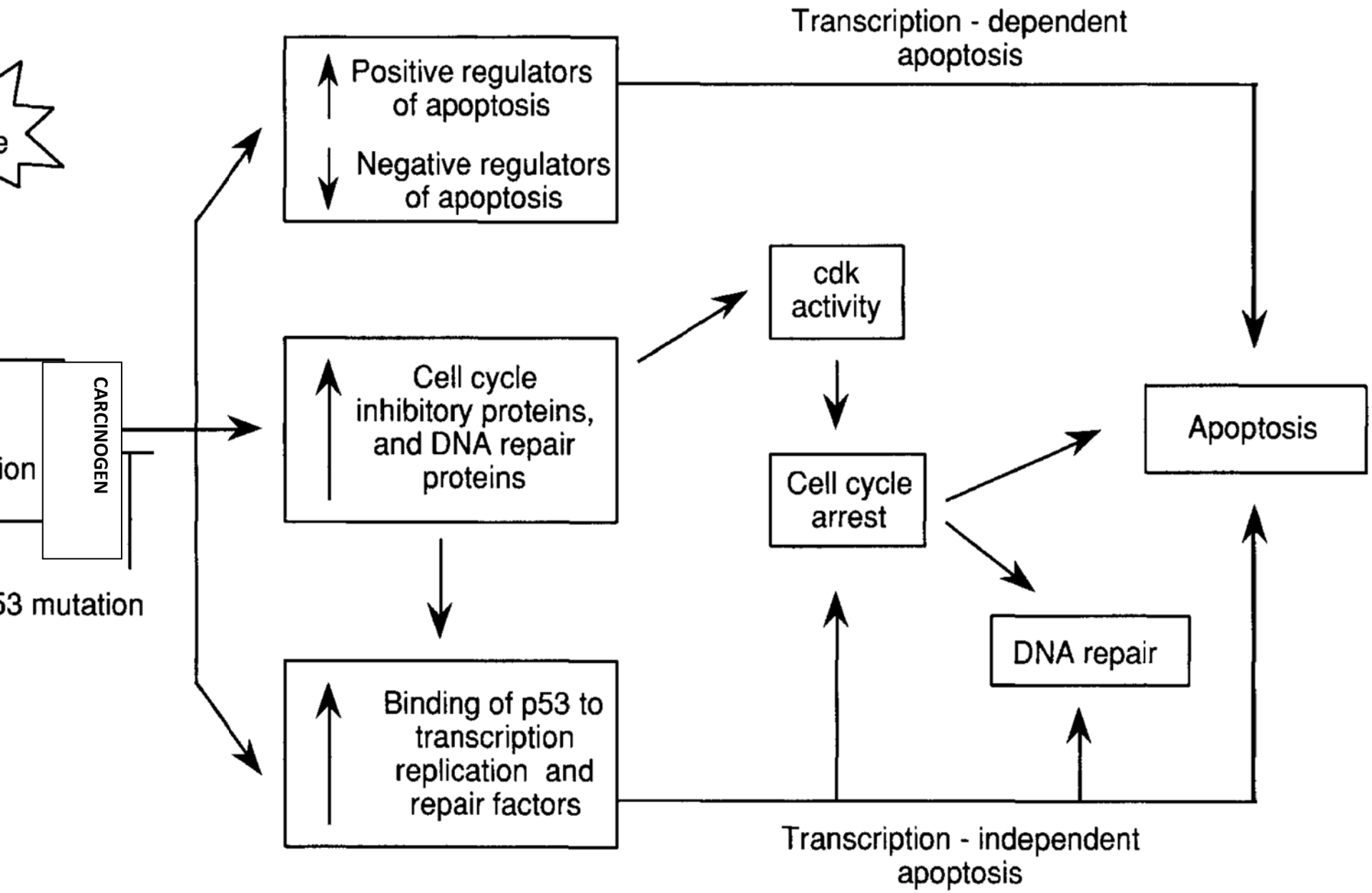


After the restriction point, the cell is committed to DNA replication and cell division. Thus, the interference with normal signal transduction pathways by chemical carcinogens, regardless of mechanism, can force a cell into proliferation that is not governed by normal physiological controls.

Even after passing through the restriction point early in G_1 phase and committing to replication, there are still multiple mechanisms through which the cell regulates progression through the cell cycle. For example, the cell must pass through what is known as a " checkpoint" at the G_1/S boundary. The G_1/S checkpoint serves to insure that DNA has been sufficiently repaired before new DNA is synthesized. The p53 protein plays a critical role at the G_1/S checkpoint. There is evidence that p53 is directly involved in the detection of several types DNA damage. Upon detecting damage, p53 regulates the production of proteins that function to bring a halt to the cell cycle. There is also evidence to suggest that p53 actually mediates the repair of certain genetic lesions by DNA repair enzymes. Once the damaged DNA has been sufficiently repaired, the cell proceeds with the synthesis of new DNA. In this phase of the cell cycle, alterations in the fidelity of DNA synthesis or inefficient repair of replication errors could have detrimental effects on the cell. Following S phase, cells pass through another

checkpoint to ensure that the DNA has been fully replicated before moving into the G_2 phase. During this phase, the cells where prepares for mitosis by checking the DNA for replication errors and ensuring that the cellular machinery needed in mitosis is functioning properly. Following G_2, the cells undergo mitosis and a daughter cell is created. Any errors in the made in the replication of the DNA of the original cell are now fixed in the DNA of the daughter cell.

If a cell has sustained an unacceptable level of DNA damage, or in situations where the cell receives irregular growth signals, such as in the overexpression of the transcription factor and protooncogene *myc*, p53 can mediate a process called *apoptosis*. Simply put, apoptosis is cell suicide. Apoptosis is an extremely important component of many physiological processes relating to growth and development. In the developing embryo, for example, apoptosis is responsible for the elimination of superfluous cells that must be eliminated to ensure proper tissue structure and function (e.g., digit formation in developing limbs). Apoptosis is also responsible for the maintenance of the correct number of cells in differentiated tissues and the elimination of cells that have been irreparably damaged. Apoptosis is an orderly process characterized by several morphological stages, including chromatin condensation, cell shrinkage, and the packaging of cellular material into apoptotic bodies (also known as blebing) that can be consumed by phagocytes in the vicinity of the cell. This orderly and well-regulated process is a distinct contrast to cell death by necrosis. As indicated previously, the p53 protein has been implicated in apoptosis resulting from several different types of cell stress, including DNA damage induced by chemical mutagens . The mechanisms by which p53 mediates apoptosis are currently a subject of intensive study for cell biologists. Some functions of p53 in the apoptotic pathway are mediated by the transcription of certain genes (e.g., *bax*) that regulate apoptosis, while other effects appear to stem from protein–protein interactions with other intercellular mediators of apoptosis.


Transcription - dependent apoptosis
Positive regulators of apoptosis
Negative regulators of apoptosis
cdk activity
Cell cycle inhibitory proteins, and DNA repair proteins
Apoptosis
Cell cycle arrest
DNA repair
Binding of p53 to transcription replication and repair factors
Transcription - independent apoptosis
CARCINOGEN
ion
53 mutation