Carcinogenesis

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Abstract

Cancer is a complex disease with multiple causes. Many intrinsic and extrinsic factors influence the development of cancer. Intrinsic or host factors include age, sex, genetics, immune system, metabolism, and hormones. Extrinsic factors are divided in different groups, as physical (different types of non-ionizing and ionizing radiations); chemical (as some mineral or organic substances); and biological (produced by some living organisms, for instance, some plants, virus, bacteria or fungi). Intrinsic and extrinsic factors can interact with one another to influence the development of cancer. In this article, we will discuss all the varied aspects of research that will ultimately lead to the prevention of cancer in man.

Keywords

Anaplasia; cancer; carcinogenesis; dysplasia; epigenetics; hyperplasia; initiation; metaplasia; metastasis; mutation; neoplasia; oncogene; progression; promotion; transduction; tumour.

Keypoints

- Providing a summary and promoting a better understanding of the carcinogenesis.
- Giving a multidisciplinary and current approach to tumorigenesis, namely on its nomenclature, molecular and cellular biology and histopathology.
- Co-relating the aspects of the biology and development of tumors with current diagnostic and therapeutic strategies.
- Illustrating the developed ideas with well-studied tumour lesions and recent scientific discoveries.

Overview

Cancer, or neoplasia, is a complex disease with multiple causes and influence factors. Even thought the global cancer death rates continue to decline, incidence rates are leveling off among men and slightly increasing among women, all over the world. Many intrinsic and extrinsic factors interfere in cancer development. Intrinsic or host factors include age, sex, genetics, immune system, metabolism, and hormones. Extrinsic factors are divided in different groups, as physical (different types of non-ionizing and ionizing radiations); chemical (as some mineral or organic substances); and biological (produced by some living organisms, for instance, some plants, virus, bacteria or fungi). Intrinsic and extrinsic factors interact one another to influence tumorigenesis. Besides its considerable physical and emotional suffering, cancer is associated a enormous cost in the healthcare systems, in lost productivity and medical and research expenditures. Considerable effort continues to be exerted by clinicians and researchers to understand this complex and multifactorial disease so that strategies can be developed to decrease or prevent its occurrence. Current regulatory guidelines have been crafted to reduce exposure to agents identified as potentially capable of causing cancer (Coleman, 2018).

During the past 45 years of cancer research, much information has been generated indicating that cancer is a multistep and progressive disease. This is supported by several research fields and studies, as epidemiology and population genetics, morphological and clinical studies, as well as experimental investigations in laboratory animals. Studies of biopsy and autopsy tissue samples from humans and animals, particularly experimental animal models of carcinogenesis, have provided important information about this multistep process at phenotypical levels. Then, molecular biological analyses have confirmed that neoplasias arise from the clonal expansion of a single transformed cell and that during its evolution, it accumulates epigenetics and nonlethal genetic damage, particularly in genes that regulate cell growth, apoptosis and DNA repair. Finally, imunological studies have allowed the comprehension of the complex cross-talk between cell tumors and the multiple cells present in tumor microenvironment (intratumoral fibroblasts, intratumoral adypocytes, intratumoral endithelial cells and pericytes, and mainly immune and inflammatory cells), conditioning and modulating its growth and development. The process of carcinogenesis may take months in experimental laboratory animals and years in humans. The identification of this process early in its natural history evolution enhances the success of surgical or therapeutical intervention strategies in termination the disease. By the time a neoplasia has progressed to the malignant stage and spread throughout the body, even

radiation, chemotherapy or immunomodulation combined with surgery are unlikely to result in clinical cure. The process of carcinogenesis is schematically summarized in Figure 1.

Nomenclature of Cancer (Neoplasia)

The word "Cancer" comes from the Latin, but it is originally Greek. It is a derived term for "crab", that describes the clinical appearance or infiltrative behavior of these abnormal growths (Zachary and McGavin, 2011), and because of the way as cancer often adheres to any body part, almost like the crab.

Carcinogenesis is narrowly defined as the production of carcinoma but is more commonly used in the broadest possible sense to indicate generation of neoplasias that are new and typically abnormal growths, generally uncontrolled, and becoming progressively more serious with time. Neoplasia means 'new growth' and two important terms that relate the clinical behavior and growth characteristics of neoplasias are (1) benign and (2) malignant, whose features are listed in Table 1. Basically, benign neoplasias are normally slow-growing circumscribed, localized growths frequently amenable to surgical removal with a low probability of recurrence, that do not metastasize. Malignant neoplasias have usually a more aggressive growth, are locally invasive, may metastasize (spread to other organs), and may be difficult to delimit during surgically excision.

Regarding the distinction between (1) tumor and (2) cancer, tumor broadly refers to any organ enlargement or swelling. Although tumor is a Celsus' cardinal sign of inflammation, it is often used as synonymous of neoplasia, as many neoplasias are associated to inflammation. Cancer refers to malignant neoplasia. Unfortunately, layperson frequently use tumor and cancer interchangeably alike without qualifying whether it is a benign or malignant process.

Neoplasias are classified based on (1) the cell or tissue of origin and (2) biological characteristics. There are two basic cell types that can originate neoplasias: mesenchymal and epithelial cells (Figure 2). Mesenchyme (tissues derived from the embryonic mesoderm) includes the connective tissue, blood and lymphatic vessels, muscles, cartilage and bones. Epithelial cells line the internal and external surfaces of the body, and origins major organs of the body, such as liver and lungs. Most epithelial tissues are derived from the endoderm and ectoderm germ layers of the embryo.

There are general guidelines used in the nomenclature of neoplasias. A benign epithelial neoplasia originating from a glandular tissue, or a tumor derived from nonglandular epithelium but exhibits a tubular growth pattern, is called an 'adenoma'. One or more qualifiers are added to indicate the tissue of origin and/or morphological features as in hepatocellular adenoma, thyroid follicular adenoma, or renal tubular cell adenoma. A exophytic (“growing outward”) tumor originating from an lining or covering that shows warty projections is denominated as papilloma; when papilloma grows inside a ductus can be referred as intraductal papilloma. In general, benign mesenchymal neoplasias are designated by attaching the suffix -oma to the name of the cell type from which the tumor originates, as in meningioma (meninges), hemangioma (blood vessels), and fibroma (fibroblasts).

Malignant epithelial neoplasias are called 'carcinomas’ and qualified by its histogenetic. Thus, malignant skin neoplasias composed predominantly of squamous cells, are called squamous cell carcinomas; if mainly formed by basal cells are classified as basal cell carcinomas. Malignant mesenchymal neoplasias are called ‘sarcomas.’ Examples of the latter include osteosarcoma, a malignant bone neoplasia; fibrosarcoma, a malignant neoplasia of the fibroblasts; and leiomyosarcoma, a malignant neoplasia of the smooth muscle tissue. A cancer composed of cells of unknown tissue origin, is designated as undifferentiated malignant tumor. Nomenclature for several neoplasias is presented in Table 2.

Neoplasia nomenclature has numerous exceptions. Though some neoplasias name have the suffix -oma they are always malignant, as the thymoma (also called malignant thymoma or thymic sarcoma), the lymphoma (malignant lymphoma or lymphosarcoma), and the melanoma (malignant melanoma). Moreover, the suffix -blastoma highlights the indiferentiation of the cells, malignancies in primitive or precursor cells, as the retinoblastoma or the nephroblastoma. On the other hand, some neoplasias are named for their physical attributes such as pheochromocytoma (in Greek, “phios” means dusky, “chroma” means color). In addition, some neoplasias may be denominated by the name of the person first describing the lesion, and examples such as Hodgkin’s lymphoma, Burkitt lymphoma, Wilms’ tumor (nephroblastoma), Kaposi´s sarcoma or Ewing sarcoma, among others, have persisted to date. Neoplasias composed of mixtures of cells are named accordingly; examples include fibroadenoma, adenosquamous carcinoma, and carcinosarcoma, mixed carcinoma of the dog´s mammary gland or the metaplastic carcinoma of the breast. Moreover, there are several tissue alterations that are not usually neoplastic in origin but have names with the the suffix -oma: hamartomas (a disorganized aggregate of normal indigenous cells that represent faulty differentiation during

embryonic development) and choristomas (focal collections of normal but ectopically located tissue due to developmental malformation, such as islands of pancreatic cells in the wall of the stomach). Finally, localized overgrowths of excess of skin with a fibrovascular axis (acrochordon) or excess inflammation and granulation tissue, such as on vocal cord or external auditory canal inflammatory polyps, are clinically recognized as pseudotumors but they are not neoplastic growths (Berman, 2004, 2005; Damjanov, 2009).

Tissue Changes Associated with Carcinogenesis

Hyperplasia and Preneoplastic lesions

Proliferative lesions, which may be classified as hyperplasia, metaplasia, dysplasia, benign neoplasia, or malignant neoplasia, represent continuous changes with considerable overlap biological and molecular features rather than discrete morphologic entities (Figure 3). The classification of lesion as preneoplastic, benign neoplasia, or malignant neoplasia depends on the lesion's most prominent morphologic and behavioural features. All neoplasias are derived from the clonal proliferation of a single initiated cell, a genetically mutated but phenotipically normal cell. Usually at some point early in the clonal expansion, the proliferating cells become phenotypically distinguishable from the surrounding normal tissue and are classified as 'preneoplastic.'

Preneoplasia refers to an increase in proliferative lesions that lead to accumulation of genotypic errors and phenotypic changes that confer the cell adaptative and selective advantages, leading to tumor development. Although not all neoplasias exhibit a morphological recognizable preneoplastic change, in those instances in which alterations are confirmed, their occurrence documents that there is a response to tissue insults. Examples of preneoplastic lesions are presented in Table 3. Preneoplastic lesions have the capacity and propensity to reversibility. In some instances, a preneoplastic lesion represents the clonal expansion of a cell that has sustained genetic damage.

A benign neoplasia is generally a localized expansive growth that compresses adjacent non-neoplastic ("normal") tissue but is usually not immediately life threatening unless it physically interferes with normal function, for example, by blocking the intestinal tract or compressing vital areas in the brain or heart. A benign neoplasia, the clonal expansion of cells that have suffered

some genetic mutations is further along the spectrum of changes that may precede the development of malignant neoplasia. In experimental carcinogenesis animal models, malignant neoplasias are frequently observed, arising from or within a benign neoplasia. Features of benign neoplasias are listed in Table 1.

Malignant neoplasias are rapidly growing, locally invasive proliferations that destroy surrounding tissues and are thus life threatening. They also may spread to distant organs in the body, mostly via the blood and lymphatic vessels. When precursor lesions are present prior to or concomitant with malignant neoplasia, it is probable that the malignancy is a consequence of the same factors that produced the precursor lesions. Malignant neoplastic features are listed in Table 1 (Brambilla *et al.*, 2003; 'Preneoplastic Changes', 2011; Feo, 2011; Zachary and McGavin, 2011; Kumar, Abbas and Aster, 2014).

Metaplasia and Dysplasia

In addition to hyperplasia, several qualitative cytological features allow the morphologic classification of the spectrum of proliferative lesions that may be observed in the process of carcinogenesis: metaplasia and dysplasia.

Metaplasia is the substitution, in the post natal life, of a fully differentiated cell type to another fully differentiated cell; although most of the times reversible, metaplasia predisposes to certain forms of neoplasia. A classic example is the replacement of the normal respiratory epithelium of airways by squamous epithelium (Figure 4) in cases of chronic lung irritation in tobacco smokers. While the squamous epithelium is believed to provide functional protection against the irritant properties of the smoke, the loss of the ciliated columnar epithelium results in increasing lung inflammation. When the irritative factor is removed, the squamous epithelium is replaced by normal ciliated columnar epithelium. Exceptionally, some metaplastic lesions, once detected, evolves to a tumor lesion, as the intestinal metaplasia of the stomach, associated with a *Helicobacter pylori* infection (Giroux and Rustgi, 2017).

Dysplasia is defined as abnormal growth of a tissue with respect to shape, size, proliferation and organization of the cells. Normal cell-to-cell orientations are disorganized or disrupted, and cells show cellular and nuclear pleomorphism, overcrowding, and increased mitosis (Figure 4). When present, dysplasia may be associated with chronic irritation, may coexist with metaplasia, and

can be seen during neoplastic transformation. It is considered a preneoplastic change precursor of malignant transformation, and may be referred to as "carcinoma *in situ*" (Kumar, Abbas and Aster, 2014).

Anaplasia - a hallmark of malignancy

Anaplasia is a qualitative alteration of cellular differentiation. Anaplastic cells may bear little, if any, resemblance to non-neoplastic (normal) cells. The lack of differentiation is associated with morphologic changes such as loss of cell polarity, celullar and nuclear pleomorphism, nuclear crowding and increased mitotic rate. This feature is considered a hallmark of malignancy (Haschek, Rousseaux and Wallig, 2010).

Staging and Grading of Cancers

In human oncology, the experience from multiple years of observation on the clinical evolution of many cancers has strengthened the predicitivity of histological grades and clinical staging in prognostication. The purpose of grading and staging a neoplasia is to predict its biological behavior and to help establish an appropriate therapeutic regimen. Grading is an evaluation of morphologic microscopic characteristics based on the extent of cellular anaplasia and the degree of proliferation. Generally, neoplasias with a high degree of anaplasia, associated specific growth patterns, and high mitotic rate, some of which may be abnormal mitosis, are given a high grade of malignancy. Most grading schemes categorize neoplasias into one to three or four grades of malignancy.

Staging of a cancer, which is independent of grading, is a clinical classification based on the extent of the cancer growth and its dissemination on the body. It provides quick prognostic information, and may influences the choice of appropriate therapy. Criteria used for staging neoplasias include the size of the primary neoplasia, the degree of invasion of the surrounding ("normal") tissues, whether the cancer has spread to local lymph nodes, or to distant tissues. Thus, it is apparent that staging will have a large influence on the therapeutic approach. A small and localized breast cancer would most likely be treated by nodulectomy and possibly radiation therapy, whereas a large, infiltrative breast cancer would more likely be treated by mastectomy.

If the cancer has spread to lymph nodes or distant sites, more aggressive therapy is implemented (Hinck and Näthke, 2014; Hortobagyi, Edge and Giuliano, 2018).

The ultimate fate of cells or proliferative tissue masses is influenced by the amount of sustained genetic damage. Cells with minimal DNA damage may persist in a latent form, indistinguishable from surrounding normal cells. If such a latent cell sustains additional damage even long after the initial insult, it may then progress further along the pathway to malignancy (Figure 1). As additional genetic damage occurs, the altered cell population expands and eventually leads to irreversible uncontrolled growth that may or may not be corrected by aggressive medical intervention.

Molecular Basis of Cancer

Multistep Genetic Model of Carcinogenesis

Genetically, the multistage process involves the activation of growth-enhancing proto-oncogenes, inactivation of growth-inhibitory tumor suppressor genes, silencing of apoptotic genes and/or DNA repair genes, as well as epigenetic events that alter gene expression, genetic polymorphisms and methylation (Table 4). Cancer cells frequently contain mutations in multiple genes as well as large chromosomal abnormalities. Since their discovery, in 1989, more than 100 protooncogenes and about 15 tumor suppressor genes have been identified. Proto-oncogenes were first discovered in cancer-causing animal viruses that carried them. Intense study of these viruses, particularly by Varmus and Bishop in the 1970s, resulted in the discovery that some endogenous animal genes had been picked up by virus ancestors and incorporated into the viral genome (denominated viral oncogenes or V-onc). Soon thereafter a number of these proto-oncogenes were identified in both the animal and human genomes and later found to play a role in cancer development (Varmus, 1988; Bister, 2015).

A widely accepted multistep model of carcinogenesis proposed by Fearon and Vogelstein in 1990 serves as the framework for studies in carcinogenesis (Figure 5). By studying multiple benign and malignant colonic neoplasias from individuals with multiple tumors, they found that benign neoplasias harbored mutations in genes such as *APC*, *ras*, and *p53*, and that there were frequently multiple mutations per neoplasia, particularly on malignant ones. The model describes a progressive acquisition of mutations, and it is believed the total accumulation of

mutations (at least five to seven) rather than the order is important in the carcinogenic process. New evidence has been published to further refine this model (Fearon and Vogelstein, 1990). Recently, it has been proposed that some neoplasias are dependent on the continued activation or overexpression of a particular oncogene for maintaining malignant behavior. Others have found that some neoplasias are 'hypersensitive' to the inhibitory effects of specific tumor suppressor genes. These findings suggest that the multistage process of carcinogenesis is not simply a summation of individual effects of cancer genes but that some individual cancer genes can override the others (referred to by some as the 'Achilles heel of cancer'), and they offer new strategies for the cancer prevention and therapy.

Proto-oncogenes and Oncogenes

Among the estimated 25 000 genes in the mammalian genome, there are about 100 genes that are classified as proto-oncogenes because activation of these genes to oncogenes appears to be an essential event for the development of many, if not all, cancers. In fact, oncogenes were first discovered by studying genetic alterations in cancers. The term oncogene activation indicates a quantitative or qualitative alteration in the expression or function of the proto-oncogene.

The proto-oncogene have essential function in the mammalian genome, mainly as cell cycle and cell differentiation regulators. These genes are highly conserved in evolution which is evidenced by structurally and functionally similar genes in yeast, earthworms, animals, and humans. Since their normal function is to control how a tissue grows and develops, if they do not function properly, abnormal growth and development may occur leading to neoplasia (so, they are called oncogenes).

The appearance (phenotype) and function of a tissue is a consequence of which genes are actively producing their programmed product, typically a protein, which in turn affects the structure and function of the cells comprising a given tissue. All somatic cells in the body inherit the same complement of maternal and paternal genes. The reason that some cells form liver and produce products such as albumin, while other cells form kidney tubules and excrete substances from the body is a consequence of which genes are expressed in those cells. Liver cells do not express several critical genes that are important in kidney function, and vice versa. Specific gene expression and its effect on tissue phenotype and function are modulated by several intrinsic and extrinsic factors (Figure 6). Since the function of proto-oncogenes is to

control cell growth, proliferation, and differentiation, inappropriate expression of these genes due to mutation (oncogenes) will result on abnormally tissue proliferation and growth, promoting tumorigenesis.

Oncogenes can be activated by several different mechanisms e.g., retroviral transduction, chromosomal translocation, gene amplification, point mutation, promoter/enhancer insertion, or decreased methylation of promoters. Once activated, an oncogene will be either inappropriately expressed (e.g., production of an altered message and protein) or overexpressed (e.g., production of too much of a normal message and protein), contributing to the neoplastic multistep process. Examples of activated or amplified oncogenes detected in human and animal neoplasias are listed in Tables 5 and 6, respectively. For some cancers, the frequency of oncogene activation is relatively high, while for other cancers, the activation of known oncogenes is uncommon. Identification of specific alterations in oncogenes in certain cancers represents a first step in determining the molecular basis of cancer and can lead to the development of tailored molecular therapeutic strategies. Experimental evidences indicate that oncogene activation can be early critical events in carcinogenesis, and experimental studies with known chemical carcinogens show that they produce specific alterations in certain oncogenes, reflecting the manner in which the carcinogen chemically affects DNA (Kontomanolis *et al.*, 2020).

Tumor Suppressor Genes

Tumor suppressor genes, originally called antioncogenes, control cell growth and differentiation, so their function suppress the development of cancerous growth. While oncogenes must be activated to promote cancer, tumor suppressor genes must be inactivated or lost for cancer to develop. It has been shown that loss or mutation of both alleles must occur in order to silence these genes. A well-known and extensively studied tumor suppressor gene is the retinoblastoma gene (*RB-1*). In hereditary retinoblastoma, an affected child is born with deletions of one allele of chromosome 13 containing the *RB-1* gene. A second mutation event leading to a loss or alteration of the remaining *RB-1* allele occurs while retinal cells are undergoing growth during development, and the ocular retinoblastoma, frequently present in both eyes, will occur early in life. Loss or alteration of both copies of this tumor suppressor gene is sufficient to cause retinoblastoma. Although named for the disease in which it was discovered,

alterations in the *RB-1* gene have been detected in breast, lung, prostate, and bone cancers (Dyson, 2016).

Acquisition of Mutations

The rate of mutation has been intensely studied in the carcinogenic process. Mutations in cellular DNA can arise during normal cell replication by infidelity in DNA replication (mispairing) as well as by chromosomal deletions, amplifications, or rearrangements. Considering mispairing in nucleotide bases alone, it is estimated that spontaneous mispairing during normal cell replication can occur with a frequency of approximately $1.4x10^{-10}$ nucleotide bases *per* cell division. Since there are nearly 10^{16} cell divisions *per* human lifespan and $2x10^{9}$ nucleotide base pairs *per* genome, a total of $2.8x10^{15}$ mispairings could occur over a lifetime (($1.4x10^{10}$) x ($2x10^{9}$) x 10^{16}). If each mispair led to a mutation that resulted in a cancer, a typical human would have billions of cancers in one average lifetime. Since such high estimates of mutation frequency are clearly in excess of what is observed, it is clear that evolutionary barriers on multicellular organisms (cell cycle arrest, apoptosis, limits to the number of cell divisions, cell adhesion, and asymmetric cell division) explains why cancer is remarkably rare. There are efficient mechanisms to repair DNA damage, thereby precluding successive accumulation of critical mutations. Cell proliferation is also critical for 'fixing' DNA damage since, without cell division there will be no inheritance of DNA errors. The cell has relatively efficient mechanisms to repair damage prior to cell division. In a rapid proliferating tissue, cell division can occur before the cell amend DNA errors, leading to increasing risk to develop cancer. While all of the above underscore the importance of cell proliferation in carcinogenesis, neoplasia does not occur exclusively or necessarily in tissues that have high proliferation rates. Consequently, other important mechanistic factors influence the complex process of carcinogenesis.

In 1994, Loeb *et al*. proposed that neoplastic cells likely have a higher mutation rate than normal cells (approximately $2x10^{7}$ per gene *per* cell division) and thereby this genomic instability increase the likelihood of neoplastic cells acquiring further mutations conducive to neoplasia. This is referred to as the 'mutator phenotype' (Figure 7). It suggests that early mutation in stability genes (i.e., DNA repair, mismatch repair, DNA replication, or chromosome maintenance) will lead to the mutator phenotype and further mutations contribute to tumor promotion and progression. Others argue that the mutation rate is similar between neoplastic and normal cells but the higher proliferation rate of neoplastic cells leads to mutation

accumulation. The healthy debates continue to feed our quest to prevent and cure the neoplastic process (Loeb and Loeb, 2000).

Growth Factors, Hormones, and Signal Transduction

While alterations in cellular DNA are critical in carcinogenesis, some cancer-causing agents, particularly those that are not genotoxic, play a major role in cancer development by indirectly influencing gene expression and growth control by altering signal transduction. While the pivotal role of hormones in the orchestration of tissue growth and development has been appreciated for decades, the recent discovery of polypeptide growth factors has added to our knowledge a complex constellation of control mechanisms that regulate normal cell growth and may cause pathogenic effects when signaling mechanisms are disturbed. Both hormones and growth factors bind to specific cellular receptors triggering cascades of intracellular signaling transducers meditors that regulate the expression of certain genes, affecting cell crosstalk and functions, as cellular proliferation. These cascades of intracellular reactions, sometimes referred to as signal transduction, are the processes whereby external stimulus triggers intracellular biochemical cascades that act as direct transcriptional regulators (activators or repressors) of specific genes. A simplified depiction of the interaction of hormones and growth factors in cell signaling is presented in Figure 8. This concept is perhaps best exemplified by the process whereby a normal hormone stimulates a tissue to grow. An example is breast development and milk production in response to the hormone prolactin. In this example, prolactin binds to a specific prolactin receptor on the external surface of the cell, which, in turn, triggers a biochemical change inside the cell membrane via molecules that are attached to the external receptor and pass through the cell membrane. This triggers a signaling cascade that mediate the activation of specific genes that initiate breast cells proliferation and milk secretion. The signaling pathways are highly interactive with numerous positive (signal-sending) and negative (signal-blocking) feedback loops. An appropriate balance between these loops is necessary for the proper functional response to the initial stimulus, and when disrupted may cause multiple diseases or even cancer.

Some forms of cancer development are believed to be facilitated by dysregulation in one or more signal transduction pathways. Thus, exposure to certain agents may potentially affect the balance of positive and negative feedback loops in one or more signal transduction pathways turning cells more susceptible to stimuli that promote growth. An example is the nongenotoxic

skin tumor promoter phorbol ester, which activates protein kinase C (PKC), a multifunctional protein kinase family that is involved in controlling the function of other proteins, playing important roles in several signal transduction cascades, many of which are critical cell regulators. Treatment of initiated mouse skin with phorbol ester activates PKC, resulting in the development of benign and malignant skin neoplasias. The complexity and pivotal importance of the signal transduction pathways help explain why multiple types of agents influence carcinogenesis, why multiple steps are involved in the carcinogenic process, and why different cancers are so heterogeneous. Signal transduction involves shifts in intracellular ion fluxes for elements such as sodium, potassium, and calcium. It also often involves activation of PKC, enzymes that phosphorylate many proteins that may be important in mitosis. Part of the signal transduction cascade involves increased expression of cyclic adenosine monophosphate, now recognized as a mitogenic signal, and activating one or more cellular proto-oncogenes. Current research demonstrate that increasing numbers of proto-oncogenes and growth factors are integral parts of the signal transduction pathways and, when altered, influence the development of cancer by subverting signal transduction (Bafico and Aaronson, 2003; Griner and Kazanietz, 2007).

Telomeres and Telomerase

Telomerase activation appears to be a critical component of the immortalization process in neoplastic cells, and it may provide the basis for new therapeutic targets. Telomeres are specialized structures at the ends of chromosomes, and telomerase is the enzyme that maintains the length of the telomeres. During each round of cell division, there is a loss of a small number of nucleotides, causing progressive erosion of genetic material at the end of each chromosome: so, as a normal cell divides, the telomeres shorten and telomerase is inactive. After a certain number of divisions, the shortened telomeres signal the cell to cease dividing and the cells become ‘senescent’ or perhaps will die by apoptosis. Germ cells and some neoplastic cells have sustained function of the telomerase enzyme, which helps maintain lengthening of the telomeres and promote continued replication. Tumors having an increased telomerase activity suggest a direct effect, but it is only part of the story. For example, p53 is activated by telomerase and in the absence of p53 these cells fail to undergo apoptosis and go on to proliferate (Corey, 2009; Jafri *et al.*, 2016).

Heredity and Cancer: Family Cancer Syndromes

That certain cancers occur in greater frequency within families represents primary empirical evidence for susceptibility based on some hereditary element. Some genetic predispositions exist for cancers of unknown etiology, while interactions between genetic susceptibility and environmental factors are probably responsible for a large proportion of human cancers. Hereditary predispositions include DNA repair deficiencies, inability to detoxify carcinogens, and germline loss or mutations of critical genes. Examples of genetic predispositions to cancer are listed in Table 7 and include neurofibromatosis, retinoblastoma, breast cancer, and colon adenomatous. In many of these instances, one event in the carcinogenic process is believed to be an inherited germline mutation in the DNA. Another inherited anomaly, a mutation in DNA repair genes causes inability to repair ultraviolet light-induced DNA damage in individuals with the condition Xeroderma pigmentosum, causing high sensitivity to sunlight exposure and a high incidence of skin neoplasia even at young ages. Individuals bearing DNA repair genes mutation have high risk to develop all cancer types as these repair systems are essential for the maintenance of genome integrity in the face of replication errors, environmental insults, and the cumulative effects of age. However, the majority of genetic damage associated with carcinogenesis is acquired either *in utero* or from environmental and/or lifestyle factors to which individuals are exposed. Even for those individuals with a hereditary predisposition to neoplasia, additional DNA damage is necessary to lead ultimately to its development. Environmental factors that may increase the risk of cancer development in genetically predisposed individuals include exposure to radiation and agents that stimulate cellular proliferation. Experimental systems to study genetic susceptibility to cancer are critically needed to assess the role of gene-environmental interaction in the development of human cancer (Axilbund, Gross and Visvanathan, 2011).

For some cancers in genetically predisposed individuals, the data are consistent with an association between malignant neoplasia and biallelic genetic alteration, and this is supported by studies of tumor suppressor genes, which prevent the development of neoplasia. Alteration or loss of a single tumor suppressor gene allele is usually insufficient to allow the development of a neoplasia. In other words, the remaining functional tumor suppressor gene copy is sufficient to prevent the development of neoplasia; if it is lost or altered, however, neoplasia can develop. This situation occurs in hereditary childhood retinoblastoma, a malignant neoplasia of the retinal cells of the eye. Susceptible individuals inherit a partial loss of one copy (one allele) of chromosome 13, where the *RB-1* is located, and acquire an alteration or loss of the remaining

RB-1 allele during early development. The affected child subsequently develops retinoblastoma, often within the first two years of life (Fabian, Rosser and Sagoo, 2018).

The Immune System and Cancer

The proper functioning of the immune system is evidenced by recovery from common childhood diseases such as mumps and chicken pox. A properly functioning immune system recognizes the foreignness of the agents responsible for these diseases, responds to and eliminates the foreign agents, and confers long-term immunity to subsequent infection by the same or similar agents. It has been proposed that cancer cells are recognized as foreign and that the immune system functions to eliminate such cells before they are transformed into large, malignant neoplasias. This process involves elaboration of antibodies that bind to the cancer cells and activate processes whereby the cancer cells are killed. In addition, specific cells of the immune system, such as cytotoxic T lymphocytes, natural killer cells, and macrophages, have mechanisms to recognize and eliminate foreign cells. The process of immune surveillance is facilitated when the cancer cells express surface antigens that are recognized as foreign. The development of malignant disease might be seen as a failure of immune surveillance and associated to immunoediting. During the escape phase of immunoediting there is an immunosupressive microenvironment that promotes tumor growth, survival, invasion and drug resistance. Exposure to agents that depress the normal functioning of the immune system can lead indirectly to neoplasia by permitting early persistence and development of recently emergent cancer cells. Once a neoplasia has reached a critical size and growth rate, it may not be possible for even a properly functional immune system to effectively eliminate the neoplastic cells, due to immune tolerance. The pharmacologic manipulation of the immune system can ameliorate cancer patients. This is the case of immunotherapy, where the use of BCG (Bacillus Calmette Guerin) in bladder cancer patients, to achieve a non-specific immune system stimulation, is a classical example. More recently new precision medicine techniques based on personalized biological therapies, as the T-cell transfer therapies (CAR T-cell therapy; TIL therapy), or dendritic cell-based vaccines are being tailored successfully in some tumor types, bringing new hope to control immunotolerence induced by tumor cells (Zhang *et al.*, 2017; Guallar-Garrido and Julián, 2020).

Operational Phases and Theoretical Aspects of Carcinogenesis

In addition to being complex, the process of carcinogenesis is typically long and tumors becomes clinically appearent just on late stages of its natural history. While perturbations in cellular DNA are essential to carcinogenesis, they alone are not sufficient to cause. Thus, in some experimental situations, a few minutes of exposure to a carcinogen is sufficient to result ultimately in cancer, whereas in other situations, exposure to the same carcinogen will not result in cancer unless there is additional experimental manipulation. Smokers illustrate this principle since many, but not all, ultimately develop lung cancer. In other experimental studies, simultaneous administration of a carcinogen and a second agent may enhance, reduce, or block the carcinogenic process depending on the agent employed. These and other carcinogenesis studies have elucidated some of the mechanisms and factors that influence carcinogenesis, delimited some of the specific stages in the multistep process, and continually reminded us of the complexity of this disease process.

Multistep experimental models of carcinogenesis are useful in defining events in the neoplastic process; provide the foundations for current operational descriptions and hypotheses of the biological mechanisms of carcinogenesis (Figure 1); are available for many organs including the skin, liver, urinary bladder, lung, intestine, mammary gland, prostate, and pancreas; and frequently are derived from chemical carcinogenesis studies on laboratory animals. The operational phases of carcinogenesis include initiation, promotion, progression and metastization (Figure 9) (Gatenby and Vincent, 2008).

Initiation

Carcinogenesis may initiate by the action of biological, physical or chemical agents that cause a non lethal, permanent, DNA error on the cell. This DNA irreversible change may ultimately cause tumor transformation, if the mutated cell do not repair the DNA damage. The initiated cell is phenotipically normal, but genotipically different from the other “normal” cells and the capacity for autonomous growth may remain latent for weeks, months, years or decades. Direct-acting carcinogens are electrophilic reactives and interact directly with nucleophilic cellular regions, as the DNA, to produce the damage while indirect-acting carcinogens must be bioactivated by the cell (metabolic activation) to produce an eletrophilic reactive intermediates that interacts with DNA exerting genotoxic damage. The majority of damaged cells have the ability to repair the

damaged DNA over a period of days or weeks; however, if a cell undergoes cell division prior to repair the DNA damage, the DNA error becomes 'fixed,' is no longer reparable, and is inherited by all subsequent daughter cells. The operational phase of initiation is relatively short and may occur within hours or days. In contrast, the promotion and progression of an initiated cell to a fully malignant neoplasia is a multistep, long process requiring months in animals and years/decades in humans. As most initiators are genotoxic, a battery of shortterm mutagenicity tests in bacteria and cell culture systems has evolved to identify chemicals with genotoxic properties, as the Ames Test. Once identified, such chemicals should be rigorously regulated to prevent human exposure. This approach is considered prudent because of the irreversible nature of the changes that occur during initiation. Indeed, it is generally believed that even a single molecule of a mutagenic agent is sufficient to damage DNA irreversibly. Thus, for practical purposes, there is no threshold or safe level of exposure to a mutagenic agent. Features of initiation are listed in Table 8.

Initiators interact with host cellular macromolecules and nucleic acids in specific patterns. Some agents have both initiating and promoting activities (see below) and can induce neoplasias rapidly and in high yield when there is repeated or high-level exposure: these agents are complete carcinogenic agents.

Cancer stem cells are a subpopulation of neoplastic cells responsible for tumor initiation and growth, and clone heterogeneity. There are rare cells with indefinite potential for self-renewal that drive tumorigenesis. Like other stem cells, they are able to develop signaling pathways during initiation and propagation. In most cases, these cells are responsible for radio and chemotherapy resistance and have a high plasticity, presenting various functional and phenotypic appearances. This diversity allows an adaptation to distinct environments and tissues and is remarkably important in the therapy and prognosis of the patient (Bajaj, Diaz and Reya, 2019; Walcher *et al.*, 2020).

Promotion

Promotion is classically considered a stage on the multistep carcinogenic process in which specific agents, known as promoters, enhance the development of pre-neoplastic stages and neoplasia by providing initiated cells with selective growth advantages over the surrounding normal cells. The features of promotion are listed in Table 8. The promoter activity on the

initiatiated cell is epigenetic, reversible but cumulative (dose-dependent effect), requiring multiple exposure of the initiated cell to the promoter agent to produce cancer. Promoters have no effect when the organism has not been previously exposed with an initiator.

Promoters are often specific for a particular tissue or species due to their interaction with receptors that are present in different amounts in different tissue types. Radiation, dietary foods or contaminants, environmental toxins, multiple medical drugs, virus and other biological agents, may act as promoters.

The temporal sequence of promoter exposure is critical for the definition of promotion. The agent must act after initiation and enhance the neoplastic process to be considered a promoter. If an agent is given simultaneously with an initiator and results in enhancement of development of neoplasias, it is regarded as a cocarcinogen rather than a promoter. While some promoters are cocarcinogenic (e.g., phorbol esters), not all promoters (e.g., phenobarbital and phenol) possess cocarcinogenicity and, conversely, not all cocarcinogens are promoters. Under these same conditions of simultaneous administration, a diminution in the neoplasia response is considered evidence of anticarcinogenic activity. Several rodent liver tumor promoters, which are active when administered after a variety of initiators, prevent or delay the development of liver neoplasias when added to diets along with an active carcinogen.

While upper and lower thresholds have been demonstrated experimentally for promoters, some consider that, in an absolute sense, it is statistically impossible to prove or disprove the existence of thresholds for promoters for much the same reasons that this cannot be done for initiators. One can never be certain that an apparent no-effect level would, indeed, be without effect if a sufficiently large enough number of animals were used. Promoters include agents such as drugs, plant products, and hormones that do not directly interact with host cellular DNA (are not genotoxic) but somehow influence the expression of DNA (epigenetic effect). Experimental evidence suggests that regulation of gene expression is unique to the nature of the promoter agent. Some promoters are believed to produce their effect by interaction with receptors in the cell membrane, cytoplasm, or nucleus (e.g., hormones, dioxin, phorbol ester, and polychlorinated biphenyls). Alternatively, promoting agents may exert their effect through their molecular orientation at cellular interfaces. Other promoters may selectively stimulate DNA synthesis and enhance cell proliferation in initiated cells, thereby giving them a selective growth advantage over surrounding normal cells; or may induce neoplasia inducing cycles of necrosis and repair (cell proliferation).

Promoters appear to have a relatively high tissue specificity. Thus, phenobarbital functions as a promoter for rodent liver neoplasia but not urinary bladder neoplasia. Saccharin, on the other hand, promotes urinary bladder neoplasia but not liver neoplasia in the rat. Similarly, 12-o-tetradecanoylphorbol-13-acetate (phorbol ester) is a potent skin and forestomach neoplasia promoter in the laboratory rodent, but has no appreciable activity in the liver. Other agents, such as the antioxidants 3-*t*-butyl-4-methoxyphenol and 2,6-di-*t*-butyl-4-methoxyphenol, may act as promoters in one organ and antipromoters in another and have no effect in a third organ. Thus, the practical definition of a promoter must include the designation of the susceptible tissue (Rao *et al.*, 1984; Matsuoka *et al.*, 1990; Stahl *et al.*, 2005).

Tumor promotion may be modulated by several factors such as age, sex, diet, hormone balance and genetic polymorphisms. The correlation of increased rates of breast cancer in women following a 'Western' lifestyle has implicated meat and fat consumption as playing an important role in breast cancer development. Experimental demonstration of the role of a high-fat diet in the promotion of mammary cancer in rats exposed to the mammary carcinogen dimethylbenzanthracene has been documented. Similarly, bile acids, as modulated by fat consumption, are known promoters of rat liver carcinogenesis and human colorectal cancer. Age and sex-associated modulations in hormonal levels of estrogens, progesterone, and androgens have been implicated as potential promoters of breast cancer on the basis of epidemiological studies in humans. Experimental studies have repeatedly shown that these hormones, in addition to pituitary prolactin, promote mammary cancer in rats initiated with carcinogens (Zhao *et al.*, 2013; Nguyen *et al.*, 2018).

Progression

Progression is the part of the multistep neoplastic process associated with the development of the cell into a biologically malignant cell population. Progression is frequently used to signify the stages whereby a benign proliferation becomes malignant or, alternatively, where a neoplasia develops from a low grade to a high grade of malignancy. During progression, neoplasias show increased invasiveness, develop the ability to metastasize, and show genetic instability, and alteration on biochemical, metabolic, and morphologic characteristics.

Tumor cell heterogeneity is an important feature of tumor progression, and includes production of antigenic and protein product variants, ability to secrete angiogenic and growth factors,

emergence of chromosomal variants, epithelial-mesenchymal transition and development of metastatic capability, alterations in metabolism, and a decrease in sensitivity to radiation or chemotherapy. The development of intraneoplastic diversity may result from increasing genetic instability. Alternatively, the heterogeneity observed in tumor progression may be generated by epigenetic, regulatory mechanisms that are a part of the process of promotion. More than likely, genetic and epigenetic events subsequent to initiation operate in a non-mutually exclusive manner during progression, possibly in an ordered cascade of latter events superimposed upon earlier events.

The most plausible mechanism of progression invokes the notion that, during the process of tumor growth, there is a natural selection that favors enhanced growth of subpopulations (clones) of the neoplastic cells. In support of this mechanism is increased phenotypic heterogeneity observed in malignant but not in benign neoplasia. Presumably, a variety of subpopulations arises, and it is only a matter of time before the emergence of a subpopulation with a more agressive biological characteristics or at least an accelerated growth advantage. This can be observed occasionally during experimental hepatocarcinogenesis when a phenotypically distinguishable carcinoma can be observed arising within an existing adenoma.

Distinction between tumor promotion and tumor progression is not readily discernible in the routine histopathologic evaluation of neoplasias and may be somewhat academic. What is believed to distinguish progression from promotion is the presence of structural genomic alterations in the former and their absence in the latter. Both structural genomic changes and biochemical changes associated with tumor progression cannot be defined by conventional histopathology. Established and emerging technologies centered on histochemistry, immunocytochemistry, *in situ* hybridization, identification of activated oncogenes, loss of tumor suppressor genes, gene expression, proteomic and metabolomic profiling offer promise to distinguish various stages of progression in the evolution from benign to malignant neoplasias (Arvelo, Sojo and Cotte, 2016).

Metastization

Metastization is defined as the spread of cancer cells from the primary site to other parts of the body mainly through the bloodstream or the lymph system, but also through coelomic cavities or contiguity (anatomic proximity). Nowadays, cancer metastasis is no longer interpreted as a

linear cascade of events but rather as a series of synchronous and partly coincident processes. For metastasis occurrence, many mechanisms are required: cell migration, angiogenesis, matrix degradation, evasion of host immune system and metastatic colonization (homing).

Initially, metastasis is highly influenced by the complex tissue microenvironment. Interactions between cancer cells, immune cells, endothelial cells, stromal fibroblasts as well as changes in tissue oxygen tension and the structure of the adjacent extracellular matrix (ECM) represent some of those influence factors. The hypoxia-inducible factor (HIF) is a potent angiogenic factor, and can switch on ameboid cell migration as the oxygen tension oscillates, stimulating mutual signalling between mesenchymal stem cells and cancer cells which leads to the metastatic phenotype. Tumour-associated macrophages (TAMs) can be stimulated by cancer-cell-secreted lactate to start angiogenesis.

Colonization of different tissues by disseminated tumour cells is a very inefficient process. Relatively high numbers of circulating tumor cells (CTCs) are detected in cancer patient's blood, but fewer metastasis are clinically detectable. After arresting in the vascular bed, a successfully metastasized cell has to arrive, subsist, and adapt in a new tissue microenvironment that may or may not be compatible with survival. That is the main reason why metastatic lesions are primarily detected in select organ sites (bone, liver, lung, and brain) but rarely in others (kidney, heart, and stomach). The preference of cancer subtypes for different tissues as a metastatic site or homing is not entirely understood yet. There are multiple theories to justify the homing process, and distinct approaches are trying to identify the processes and factors involved in tumour colonization. For instance, the metastasis suppressor *RARRES3* facilitates breast cancer tropism to the lung by increasing the lung parenchyma's cellular invasion. Circulating growth factors and cytokines as well as microRNA exosomes, have been described to contribute to the modification of premetastatic niches, and changes on tumour microenvironment and drug resistance. Furthermore, animal model studies have contributed to understanding their effects on increasing vascular permeability and inducing alterations on the metastatic site's resident cells. Recent reports have also suggested the role of specific integrin receptors on the exosomes in tissue tropism. Thus, metastatic colonization is not merely an outgrowth of cancer cells from the primary organ but a group of complex interactions between disseminated cancer cells and the different tissue microenvironments of the organism (Seyfried and Huysentruyt, 2013; Arvelo, Sojo and Cotte, 2016; Suhail *et al.*, 2019).

Exogenous Factors Influencing Carcinogenesis

Important exogenous factors that contribute to induction of cancer include natural and synthetic chemicals, environmental exposures to ultraviolet and medical radiation, diet and lifestyle, and infectious agents such as viruses, parasites, and bacteria. Evidence for a causal association between exogenous factors and neoplasia is derived from epidemiologic studies, analysis of occupationally exposure common cancers, and animal models.

Chemical and Physical Agents and Lifestyle Factors

Many chemicals that cause cancer interact directly with and alter DNA or are metabolized to chemical derivatives capable of doing so. Exposure to carcinogens can occur in certain occupational settings. Association of hepatic angiosarcomas with occupational exposure to vinyl chloride, pulmonary mesotheliomas with exposure to asbestos fibers, and leukemia with benzene are well-known examples. Exposure to other carcinogenic agents may occur in the diet or as a consequence of certain lifestyle practices such as cigarette smoking associated with pulmonary cancer and high animal fat diets linked to breast and colon cancer. Strong associations have been made between exposure of light-skinned individuals to ultraviolet radiation and skin cancer. Exposure to natural or occupational ionizing radiation, X-rays, and medical radioisotopes, medical drugs and chemotherapeutic drugs have also been associated with human neoplasia. Examples include leukemias in radiologists and atom bomb victims, lung cancer in uranium mineworkers, and thyroid and breast cancer following diagnostic or therapeutic use of radiation, and bladder cancer in paint or rubber industry workers (Charbotel, Fervers and Droz, 2014; Mundt *et al.*, 2017).

Infectious Agents and Inflammation

Viral, parasitic, and bacterial infections have been linked to cancer (Table 9). DNA viruses such as Epstein-Barr, hepatitis B, hepatitis C, papillomaviruses, and Kaposi sarcoma herpes virus and RNA viruses such as human T-cell leukemia virus type I and human immunodeficiency virus have been implicated in causing cancer in humans and are listed as ‘known-to-cause-cancer’ in humans by the International Agency for Research on Cancer (IARC). In man, the liver fluke, *Opisthorchis viverrini*, is associated with the development of cholangiocarcinomas of the liver

and the blood fluke, *Schistosoma haematobium*, with carcinoma of the urinary bladder. There is evidence that chronic *Helicobacter pylori* infection of the stomach in humans not only is related to gastrointestinal ulcers, but also may be linked to gastric carcinoma or lymphoma development (van Tong *et al.*, 2017).

For oncogenic viruses, the viral or host genes generally drive the neoplastic process while for some agents there appears to be an association between biological and other physical or chemicall carcinogenetic mechanisms as in the chronic inflammation and nitric oxide (NO) production in the development of cancer. When DNA viruses infect cells, the viral DNA inserts itself wholly or partially into the genome of the infected cell. It appears that such integration of viral DNA into the mammalian genome is sometimes sufficient to cause neoplastic transformation of the infected cell, which is accompanied by the production of new proteins essential for the neoplastic process. RNA viruses associated with neoplasia are chiefly represented by the retroviruses. RNA viruses possess an enzyme called reverse transcriptase, which is capable of forming a DNA copy of the viral RNA when the virus infects a host cell. This DNA ultimately inserts itself into the host genome in much the same way as DNA viruses do, possibly resulting in the development of neoplasia (Lambert, 2009).

The role of inflammation in cancer development is being intensely studied. There are a number of chronic inflammatory conditions, infectious and noninfectious, in humans and animals associated with an increasing risk of cancer, and there are many investigators examining the role of NO and oxygen radical damage to DNA or other cellular processes, such as cell proliferation and apoptosis. NO induces p53, prevents apoptosis in cells such as endothelium, promotes angiogenesis, and inhibits DNA-repair activities - all processes that might provide a selective advantage to neoplastic cell growth (Singh *et al.*, 2019).

Identification of Carcinogenic Agents

There are two methods utilized to identify potential human carcinogens, the most direct of which is based on retrospective epidemiological studies in human populations using existing historical records associated with known cases of neoplasia. These records include death certificates where cause of death is indicated; hospital records; responses to questionnaires that document environmental or work-associated exposure to potential carcinogenic agents; and studies of neoplasia in culturally, ethnically, or religiously distinctive human populations.

Association of cigarette mesotheliomas and exposure to chemicals and bladder cancer was the result of such retrospective epidemiological work. Prospective epidemiological studies identify a given population of individuals who agree to be monitored for several years to permit identification of potential carcinogenic factors associated with neoplasias that may occur.

Another method used to identify potential human carcinogens involves testing known chemicals and agents in experimental animals. Such tests have been referred to as animal bioassays and are typically conducted using rodents (mainly rats and mice) exposed to high doses of the suspect agent for a large portion of their lifespan (typically two years). If such agents are observed to produce neoplasia in the experimental animals, the agent is regarded as a potential human carcinogen. In countries worldwide, legal requirements mandate that all new chemical agents and drugs be tested in animal bioassays to determine whether they cause cancer in the test animals. Additionally, since the mid-1960s in the United States, the National Cancer Institute and currently the National Toxicology Program have collectively conducted animal bioassays on more than 500 chemical agents to assess their potential to cause cancer.

Interpretation of results from human epidemiological studies and animal bioassays to identify carcinogenic agents has often proved difficult and controversial. Humans are rarely exposed to only one potential cancer-causing agent in their lifetime, and the amount and duration of that exposure may be difficult or impossible to quantify rigorously. Long latency intervals may occur between exposure to a potential carcinogen and ultimate development of neoplasia, making accurate assessment of cause and effect almost impossible. Despite such limitations, epidemiological studies that clearly show an association between a given chemical exposure or lifestyle habit with an enhanced rate of a specific cancer are regarded as the most relevant method for identification of human carcinogens. While animal bioassays have proved useful for the identification of agents that can cause cancer in the laboratory rodent, they only identify an agent as potentially hazardous to human health. Additional facts and factors must be considered in classifying such an agent as a likely human carcinogen (Carcinogens, 1996; Ashby, 1997).

The current approach for assessing the scientific relevance of either epidemiological or animal bioassay results to human health risk involves a ‘weight-of-evidence’ procedure in which national and international panels of expert scientists from several disciplines examine all available information on the suspect agent in making their assessment. Included in this analysis are the strength of the epidemiological evidence, the dose-response curve of the animal response, comparative species metabolism and ability to extrapolate between species, likely mechanism of cancer induction for the agent in question, its genotoxicity, the amount of the

agent in the environment, and the number of people potentially exposed to it (Madia *et al.*, 2020). Based on this type of analysis, so far 121 agents have been classified as known human carcinogens by the IARC (some of which are in Table 10) and 89 more agents have been designated as probable human carcinogens. The 14th US Health and Human Services Annual Report on Carcinogens lists 62 known human carcinogens and 135 substances that are reasonably anticipated to be human carcinogens.

Molecular Epidemiology of Cancer

The molecular epidemiology of cancer is the study of molecular alterations, primarily mutations, in investigating the etiology of cancer, as well as, identifying individual cancer risk. The possibility of identifying cancer-causing agents based on the occurrence of predictable molecular alterations that are found in the neoplasia is intriguing. It is based on the hypothesis that there are carcinogen-specific patterns of mutations that reflect direct interactions of carcinogens with cancer genes. For example, lung and colon cancers from smokers tend to have a specific mutation in the *ras* oncogene or *p53* tumor suppressor gene (i.e., mostly a G-T nucleotide base substitution) and that this mutation is likely due to the direct interaction of the carcinogen in smoke benzo(*a*)pyrene with DNA (Rivlin *et al.*, 2011). Such chemical-specific mutational profiles (or 'molecular signatures') have been used to support a causal association between particular genetic events in tumors and a specific carcinogen, such as neoplasias associated with exposure to radon, aflatoxin B1, vinyl chloride, and the nitrosamines (Tables 11 and 12). The strongest evidence for linkage between a cancer-causing agent and a specific type of neoplasia is that of the CC-TT double base changes observed in skin neoplasias of both humans and animals. This mutation is consistent with the predicted UV-induced damage of dipyrimidine dimers. In liver tumors from persons living in geographic areas with a high exposure to aflatoxin B1, there is a frequent mutation at the third nucleotide pair of codon 249 in the *p53* gene, suggesting the mutation is chemical specific and imparts a specific growth or survival advantage to the mutated liver cells.

Animal studies have confirmed that there are certain chemical-specific mutational profiles in neoplasias; however, there are many examples where the mutational profile varies by strain (Table 13), species, dose, or dosing regiment. For example, diethylnitrosamine, a strong, cross-species hepatocarcinogen, will induce liver neoplasias in mice, rats, and rainbow trout, but the frequency and type of *ras* mutation in the neoplasia vary widely, and the mutations are not

simply a reflection of direct DNA interaction (Table 14). In some studies, *in vitro* mutation assays were poor predictors of liver tumor mutation profiles in the mouse. In this complex process, carcinogens might also be influencing by events such as DNA repair, oxidative DNA damage, methylation, cell death, proliferation, and/or a hypermutable state.

Molecular epidemiologic studies aiming identify individual's risk of developing cancer have found that persons with germline mutations in cancer genes (i.e., BRCA1 or BRCA2) or genetic variations (genetic polymorphisms) of carcinogen metabolizing enzyme activities (i.e., cytochrome P450s or glutathione-S-transferases) or DNA repair capacities can present increased risk of tumorigenesis in their lifetime. High throughput analyses to examine single nucleotide polymorphisms (SNPs) are being used to search for biomarkers of cancer risk in individuals, and some of this information is being used to stablish preventive measures to decrease carcinogenic risk (Chen and Hunter, 2005).

Conclusion

All of life is a balancing act of good *versus* evil and production *versus* destruction. Similar balancing factors are evident in carcinogenesis where regulatory mechanisms for tissue proliferation are balanced against those for cellular differentiation. It is well established that carcinogenesis requires the accumulation of multiple alterations in the genome of the affected cells. At the genetic level, two opposing classes of genes, oncogenes, and tumor suppressor genes, as well as apoptotic and DNA repair genes, have been implicated in carcinogenic process. In addition, the development of cancer is influenced by host factors such as age, sex, diet, nutrition, general health status, and inherited predispositions for cancer and by complex positive and negative intracellular signaling mechanisms. Treatment of cancer is based on our understanding of the mechanistic underpinnings of the carcinogenic process and attempts to shift the balance of critical factors in favor of patient survival. The probability of developing cancer is directly proportional to the intensity, route, and duration of exposure to cancer-causing factors as well as genetic susceptibility. Public health strategies are based on the premise that reduction or prevention of exposure to cancer-causing factors will decrease the incidence of cancer.

See also: Carcinogen Classification Schemes; Carcinogen-DNA Adduct Formation and DNA Repair; Carcinogenesis; Cell Proliferation; Chromosome Aberrations; Epidemiology; Immune

System; International Agency for Research on Cancer; Mechanisms of Toxicity; Molecular Toxicology: Recombinant DNA Technology; Mouse Lymphoma Assay; Radiation Toxicology, Ionizing and Nonionizing; Skin; Toxicity Testing.

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