CHAPTER 1

THEORIES OF CARCINOGENESIS

The oldest description of human cancer, referring to eight cases of tumors of the breast, was found in the Egyptian Edwin Smith Papyrus, written around 3000–1500 BC. The oldest specimens of human cancers were detected in the remains of a female skull dating back to the Bronze Age (1900–1600 BC), and in fossilized bones of ancient Egypt. The mummified skeletal remains of Peruvian Incas, dating about 2,400 years ago, contained lesions suggestive of malignant melanoma. The term "cancer" goes back to Hippocrates (460–370 BC), who named a group of diseases καρκινοσ and καρκινομα, the ancient Greek word for crab. It is a metaphor for the hard center and spiny projections of the tumors he studied. Cancer is the Latin word for crab and its use has been traced back to Galen (AD 129–199). A snapshot of theories of carcinogenesis, devised in the course of the last two centuries, reflects the progress of insight from the cellular level via biochemistry to an understanding of damaging influences and oncogenes, and to a more wholistic approach in the regulatory theory. It shows the relative success of reductionism as well as the current need to put the insights of various research endeavors into broader paradigmatic contexts.

1.1 CELLULAR THEORIES

In 1665, Robert Hooke described walled cavities in his microscopic examination of cork and called them cells. In 1805, Lorenz Oken conceptualized a cell-based theory of life, arguing that plants and animals are assemblages of tiny living infusoria. This notion was later populated and refined by Matthias Schleiden and Theodor Schwann [Schleiden 1838; Schwann 1839; Nurse 2000]. In 1841, Robert Remak (1815–1865) described the phenomenon of cell division in chick embryos and in muscle development. Between 1850 and 1855, he extended these observations to embryonic development and proposed that tumor cells arose by cell formation from existing specific tissues [Remak 1852, 1855]. Like Giovanni Morgagni, who had performed the first autopsy in 1761 and had correlated illness to macroscopic pathology, Rudolf Virchow (1821–1902) correlated illness to microscopic pathology. After initial skepticism, Virchow acknowledged Remak's evidence for cell division. In 1858, he gave a series of 20 lectures to a group of physicians at the Institute of Pathology in Berlin, in which he summarized his experience in microscopic anatomy of tissues with special attention to those deviating from the healthy condition [Virchow 1858]. According to Virchow's dictum "omnis cellula e cellule" cells of diseased tissues are derived from normal tissues, implying that malfunction begets disease (significantly, Virchow had been a student of Müller's, who had demonstrated in 1838 that cancer is made up of cells, not lymph; but he was of the opinion that cancer cells arose from interstitial budding elements, blastema, not from normal cells). Hence, tumors are derived from cells that divide faster than they should. The average human body experiences around 10^{16} cell divisions in a lifetime. With an individual's risk to contract cancer being about 10%, malignant transformation occurs in 1 out of 10^{17} cell divisions [Weinberg 1998]. The mechanistic underpinning for this process was defined by the identification of key regulators of the cell division cycle by Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse.

The analysis of transformation has been guided substantially by the technical accomplishment to expand cells in culture. Tissue culture was developed in the early years of the 20th century [Harrison 1907; Burrows 1910]. Warren Lewis cultured rodent cancers [Lewis 1936]. In 1951 at the Johns Hopkins Hospital, George Gey established human cancer cell culture from the cervical adenocarcinoma of the 30-year-old, black Henrietta Lacks [Gey et al. 1952]. Although the resulting HeLa cells are among the cornerstones of cancer research, their high rate of proliferation caused a risk for cross-contamination of other cultures by them. This lead to the establishment of cell-typing techniques on the biochemical [Gartler 1967] and genetic [Nelson-Rees et al. 1973] levels. Generally, the human tumor cells that grow permanently in culture are a selected group of very aggressive cancers. Almost all of the continuous cell lines are derived from high-grade, high-stage cancers.

Programmed cell death (apoptosis, Greek: falling off of tree leaves) [Kerr et al. 1972] may be invoked by many organisms as a control mechanism to prevent unrestricted growth. Research during the 1960s through 1970s in the worm *Caenorhabditis elegans*, identified *ced-3* and *ced-4* as essential genes for programmed cell death, while *ced-9* was found to be a negative regulator of apoptosis. The 2002, Nobel Prize in Medicine and Physiology was awarded for these observations to Sydney Brenner, H. Robert Horvitz, and John E. Sulston. The first mammalian homolog for *ced-3* was described as *bcl-2*, a gene that is involved in B-cell lymphomata [Negrini et al. 1987; Vaux et al. 1988]. *bcl-2* transfected B-lymphocytes are resistant to apoptosis, which is typically induced by Interleukin-3 withdrawal. For the first time, it was demonstrated that the pathway to tumorigenesis depends not only on the ability to escape growth control but also on the ability to prevent cell death [Hockenbery et al. 1990].

1.2 BIOCHEMICAL THEORIES

According to the biochemical theory of cancer, a key process that governs cell proliferation goes awry and causes transformation. Various aspects of metabolism may be affected in a manner that could lead to cancer. Consequently, before the discovery of oncogenes, a large variety of theories was debated, which incriminated the malfunction of diverse biochemical processes as causative for malignant transformation.

During tumor progression, the enzymatic composition of the affected cells is simplified (described as the theory of convergence in cancer), so that various cancers resemble one another more than they resemble their tissue of origin [Greenstein 1954]. As one possible underlying reason, the biochemist Otto von Warburg [von Warburg 1930] had suggested that the oxidative metabolism in cancer cells is replaced by glycolysis and that the excessive proliferation of cancer cells reflects their ability to metabolize independently of oxygen. Later, it was found that the limiting substrates for tumor growth are oxygen and glucose. Hence, anaerobic glycolysis is not the cause, but the consequence of the accelerated growth, which cannot be satisfied by the reorganization of the micro-vasculature [Vaupel et al. 1976]. However, in a remarkable reversal toward supporting the Warburg model, a 2005 publication showed that in cells engineered to become cancerous glycolytic conversion started early and expanded as the cells became more malignant [Ramanathan et al. 2005]. This rekindled the discussion of bioenergetics in cancer cells.

Others attributed the simplified enzyme patterns of cancerous cells to a regression of the tumor tissues to early embryonal stages of development. Highly malignant cells tend to resemble fetal tissues more than their adult normal counterparts do. The idea of derepressive dedifferentiation in carcinogenesis found support in the occurrence of onco-fetal proteins during the disease. The expression of these genes should be repressed in differentiated tissues, but this repression is reversed in tumors. The description of tumor tissue in histopathologic analysis as dedifferentiated is derived from this concept. The alternative model of "oncogeny as partially blocked ontogeny" suggested that cancer is the result of a series of alterations in the genes and their gene expression, which prevent a stem cell from completing all the steps necessary for terminal differentiation, suggesting that the target cell for carcinogenesis is the pluripotent stem cell [Potter 1978].

The protein deletion theory, an extension of the dedifferentiation theory, is an epigenetic model of cancer. Based on the observation that a carcinogenic aminoazo dye covalently bound liver proteins in animals undergoing early carcinogenesis, whereas little or no dye binding occurred to the proteins of

tumors induced by this dye, Miller and Miller [1947] proposed the deletion hypothesis. They suggested that carcinogenesis resulted from permanent alterations or loss of proteins that are essential for the control of growth. Thus, carcinogens eliminate specific enzymes from the affected cells by binding covalently to water-soluble basic proteins (h_2 proteins according to electrophoresis nomenclature). This causes the elimination (deletion) of these proteins from the cells. Cancer originates because the water-soluble basic proteins contain several growth inhibitory components. Therefore, the initial step in carcinogenesis is the inactivation of endogenous inhibition.

Transformation can be associated with refraction to exogenous inhibitors of cell cycle progression. Potter [1964] suggested that the proteins lost during carcinogenesis may be involved in the feedback control of enzyme systems required for cell division, and he proposed the feedback deletion theory. In this model, repressors crucial to the regulation of genes involved in cell proliferation are lost or inactivated by the action of oncogenic agents on the cell, either by interacting with DNA to block repressor gene transcription or by reacting directly with repressor proteins and inactivating them. It was thought that experimental evidence, in which the fusion of cancerous cells with nontransformed cells resulted in the absence of transformation, supported the epigenetic theory. Later, this phenomenon was attributed to the functional dominance of tumor suppressor genes.

The demonstration of the presence of an ordered biochemical imbalance, linked to transformation and progression, in cancer cells led to the molecular correlation concept. Weber [1977] stated that the biochemical dysregulations underlying neoplasia could be identified by elucidating the pattern of gene expression as revealed in the activity, concentration, and isozyme aspects of key enzymes and their linking with neoplastic transformation and progression. Key enzymes are involved in the regulation of rate and direction of the flux of competing synthetic and catabolic pathways and are most likely affected in the malignant process. A number of enzyme activities found to be altered in malignant cells are those involved in nucleic acid synthesis and catabolism. In general, the key enzymes in the de novo pathways and salvage pathways of purine and pyrimidine biosynthesis are increased and the opposing catabolic enzymes are decreased during malignant transformation and tumor progression. These findings and concepts were further developed by the analysis of gene expression profiles and identification of gene expression signatures in cancer cells some 20 years later.

1.3 NOXIOUS THEORIES

The stigma that cancer equals death, originating in the experiences of Hippocrates, Galen, and Celsus, was attached to the disease for centuries. It led to the long-respected dictum that doctors should not inform their patients of the diagnosis to avoid agony. In view of progress in surgery, which allowed the removal of some tumors, the American Cancer Society was formed in 1913 to educate the public about the warning symptoms of cancer and to reduce their fatalistic fears. The increased public health awareness was helpful whenever carcinogenic mechanisms were identified and the need for lifestyle changes was publicized. The insight that malignancy may be caused by the influence of damaging agents forms the basis of the noxious theory of carcinogenesis. Among the influences that may cause cancer are chemicals, radiation, and viruses.

Chemical carcinogenesis. In 1775, chemical carcinogenesis was observed by the English surgeon Sir Percival Pott, who related the cause of scrotal skin cancer in a number of his patients to a common history of occupational exposure to large amounts of coal soot as chimney sweepers when they were boys. The connection between soot and cancer was confirmed in 1915 by the first controlled experimental induction of cancer in laboratory animals by Katsusaburo Yamagiwa. The experiment established chemical carcinogenesis, and specifically occupational exposure, as one possible cause for malignant growths. An unrelated form of occupational exposure was documented in the mid-19th century in silver miners from St. Joachimsthal, Bohemia (today Czech Republic). Silver had been extracted there since the mid-16th century and was manufactured into the Joachimsthaler silver coins that were predecessors of the German currency "Thaler" and later the American currency "dollar." These miners had a high incidence of lung cancer, which was otherwise extremely rare at that time. The cause was traced to their occupational exposure (Table 1.3.A).

Archeological evidence suggests that the Mayans smoked tobacco leaves as early as the 1st century BC.

Table 1.3.A. Occupational cancers. Certain occupations are associated with high levels of exposure to specific carcinogenic influences. These agents cause DNA damage through physical or chemical effects. Accordingly, the types of cancers induced by these carcinogens have a higher than normal incidence among exposed workers

Agent	Occupation	Site of cancer
X-rays	Radiologists, radiographers	Skin
Ultraviolet radiation	Farmers, sailors	Skin
Polycyclic hydrocarbons (soot, tar)	Chimney sweepers, manufacturers of coal gas	Skin, bronchus, scrotum
Asbestos	Insulation workers, shipyard workers	Bronchus, pleura, peritoneum
Radon	Underground miners for uranium or fluorspar	Bronchus
Bis(chloromethyl)ether	Ion-exchange resin manufacturers	Bronchus
Mustard gas	Poison gas manufacturers	Bronchus, larynx
Tobacco smoke	Flight attendants, bar tenders	Lung
Naphthylamine	Rubber workers, manufacturers of coal gas	Bladder
4-aminobiphenyl	Chemical workers	Bladder
Vinyl chloride	PVC manufacturers	Liver (angiosarcoma)
Benzene	Workers with glues or varnishes	Bone marrow (leukemia)
Radium	Luminous dial painters	bone
Arsenic	Sheep dip makers, gold miners, vineyard workers, ore smelters	Epidermoid and basal cells, bronchus, liver, bladder

Only in 1761, John Hill published a treatise that warned of unusual tumors of the nose consecutive to sniffing tobacco. By 1949, Ernst Wynder had conducted a survey of 684 lung cancers, which indicated a substantially elevated risk in smokers compared to nonsmokers. It was followed 6 months later by a similar analysis, authored by Richard Doll. About 188 years after the publication by John Hill, a connection between lifestyle choices and cancer risk was established. During the following years of the 20th century, chemical carcinogenesis by tobacco products became a major cause for an increasing incidence of lung cancers. (Table 1.3.B).

In Italy, Bernardino Ramazzini associated breast cancer with reproductive factors. He reported in 1713 the virtual absence of cervical cancer and relatively high incidence of breast cancer in nuns and suggested that this was in some way related to their celibate lifestyle. The key observations by Pott, Hill, and Ramazzini laid the foundation for the field of cancer epidemiology. This area of research was given another foundation between 1930 and 1932, when Fisher, Haldane, and Wright established the principles of population genetics. In the United States, the first hospital registry for cancer was established in 1926 at Yale-New Haven Hospital in Connecticut. In 1935 and 1946, the first central cancer registries were initiated in Connecticut and California. In 1941, the United States National Cancer Institute published a survey of 696 chemical compounds, 169 of which were found to be carcinogenic in animals. During the 1960s, environmental movements became prominent in most of the Western societies. Rachel Carson believed that the long-term ecological effects of synthetic chemical pesticides were not being researched adequately. Her book "Silent Spring" pointed to the pathogenic potential of environmental toxins, and the concept of carcinogens entered popular consciousness. In 1964, Rachel Carson succumbed to cancer at the age of 56. The National Cancer Act of 1971 (declared "war on cancer" by President Richard Nixon) mandated the collection, analysis, and dissemination of all data useful in the prevention, diagnosis, and treatment of cancer. It resulted in the establishment of the National Cancer Program, under which the Surveillance, Epidemiology, and End Results (SEER) Program was developed in 1973.

Over the years, the susceptibility to various cancers has been associated with nutritional habits. In 1981, Doll and Peto [1981] estimated that 35% of cancer deaths in the United States were attributable to dietary factors. The Western European diet is rich in meat and correlates with a high incidence of colon cancer. Nasopharyngeal cancer is among the most widespread tumors in Southeast Asia, possibly supported by the ingestion of salted fish. Esophageal cancer typically occurs in conjunction with alcoholism. The growing health conscience in the late years of the 20th century, combined with insights into the potential carcinogenic properties of reactive oxygen intermediates prompted multiple studies into cancer preventive capacities of antioxidants as nutrition supplements. It was soon found that while

Table 1.3.B. Chemical carcinogens. While all chemical carcinogens share the property of damaging DNA, various compounds cause the formation of tumors in diverse organs. Among many mechanisms, this may reflect the site of exposure (skin, lungs), the site of metabolism (liver), or the site of accumulation during excretion (bladder)

Chemical Compounds	Cancer
Pro-carcinogen	
Polycyclic aromatic hydrocarbons	
3,4-Benzopyrene	Lung and pancreas cancer
3-Methylcholanthrene	Lung carcinoma
Benzanthracene	Bladder and skin cancer
7,12-Dimethylbenzanthracene	Mammary carcinoma
Aromatic amines and azo dyes	
2-Naphthylamine	Bladder carcinoma
Benzidine	Bladder carcinoma
2-Acetylaminofluorene	Bladder, kidney, and liver cancer
4-Dimethylaminoazobenzene	Liver tumors
Mycotoxins	
Aflatoxins	Hepatocellular carcinoma
Mitomycin C	
Metals	
Arsenic	Skin cancer, lung cancer
Chromium (hexavalent compounds)	Lung cancer
Cadmium	Sarcomas, testicular cancer
Nickel	Lung cancer
N-nitroso compounds	
Nitrosamines	Liver cancer
N-nitroso-piperidin	Liver cancer, esophagus cancer
Nitrosourea	Intestinal cancer, squamous skin cancer
Other pro-carcinogens	
Chlordane	Liver cancer
Carbon tetrachloride	Liver cancer
Direct-acting carcinogens	
Alkylating agents	
Cyclophosphamide	Bladder cancer, skin cancer
Busulfan	Leukemia, kidney cancer, uterine cancer
Chlorambucil	
β-Propiolactone	Skin cancer, stomach cancer
Bis(chloromethyl)ether	Lung cancer
Acetylating agents	
1-Acetylimidazole	Lung cancer
Promoters	
12-Tetradecanoyl phorbol-13-acetate (TPA, PMA)	
Dichlorodiphenyl-trichloroethane (DDT)	Breast cancer
Phenobarbital	Liver cancer
2,3,7,8-Tetrachloro-*p*-dioxin	Lymphoma
Cyclosporin	Squamous cell carcinoma
Unknown function	
Vinyl carbamate	Lung cancer
4-(Methylnitroamino)-1-(3-pyridyl)-1-butanone	Lung cancer

the intake of some foods can increase the risk for specific malignancies, others – such as retinoids – can act in a chemopreventive [Sporn et al. 1976] fashion (Figure 1.3.A).

From their studies of oral cancer, Slaughter, Southwick, and Smejkal derived the concept of carcinogenesis as a process of field cancerization (field carcinogenesis, condemned mucosa syndrome). The repeated exposure of a region's entire tissue area to carcinogenic insult increases the risk for developing multiple independent premalignant and malignant foci in that tissue [Slaughter et al. 1953]. Increasingly, molecular mechanisms have been identified to link certain toxins to specific cancers. In 1975, Bruce Ames at the University of California in Berkeley developed a test for the mutagenicity of chemical compounds, which was used to confirm that carcinogens are mutagens. Further mechanistic insight was gained with the demonstration that aflatoxin causes the mutation G249T in *p53*, which is associated with hepatoma [Bressac et al. 1991]. Ultraviolet (UV) light induces pyrimidine dimers, which cause mutations in *p53* that lead to skin cancer [Brash et al. 1991; Pierceall et al. 1991].

The double-edged sword of mutagens became evident when their possible benefit in the treatment of neoplasias was discovered. Mustard gas had been used as a chemical warfare agent during World War I and was studied further in World War II. In 1917, Krumbhaar, a Captain in the US Medical Corps, noted the development of profound leukopenia in individuals who survived a gas attack for several days [Krumbhaar 1919]. Following up on this observation, a group of the US Office of Scientific Research and Development (OSRD) at Yale Medical School secretly studied the effects of nitrogen mustard on lymphomata. There, Lindskog successfully treated a radioresistant lymphosarcoma that compressed the patient's trachea with the injection of nitrogen mustard in December 1942. None of this was made public until 1946. During a military operation in World War II, allied ships in Bari harbor, Italy, were sunk in an air assault (2 December 1943). At the center of the destruction was the vessel John Harvey, laden with ammunition, supplies, and 2,000 mustard gas bombs. A large number of military personnel were accidentally exposed to mustard gas and were later found to have abnormally low white blood cell counts. It was reasoned that an agent, which damaged the rapidly growing white blood cells, might have a similar effect on cancer. Cornelius P. Rhoads served as chief of

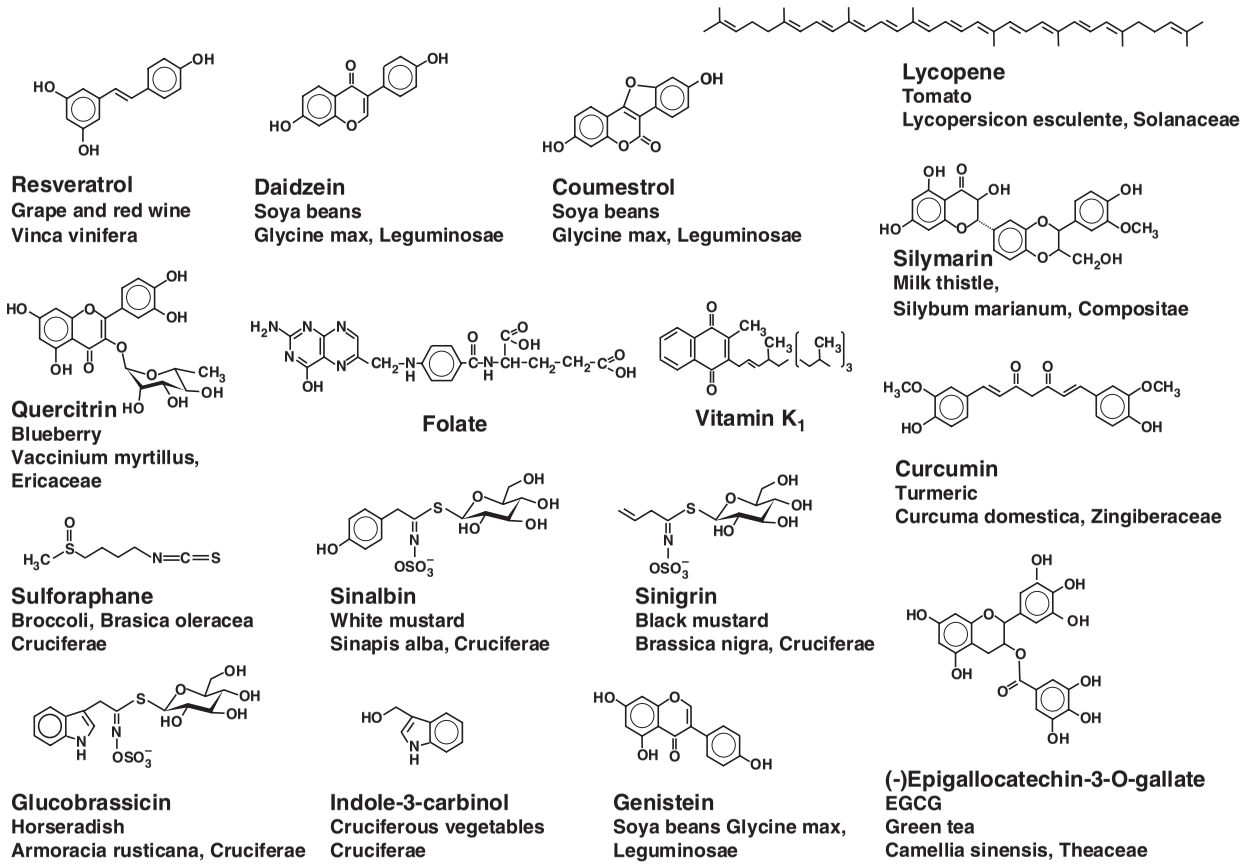

Figure 1.3.A. Dietary cancer chemopreventive compounds. Examples of compounds that have protective properties against certain cancers. Shown are the chemical structures, names, and food sources. [Reproduced from http://visiscience.com/free_powerpoint_slides.php]

the medical division of the US Army's chemical warfare unit during World War II. Based on his experience in the Bari incident, he investigated mustard gas as a tumor-killing agent. This presaged classical chemotherapy [Rhoads 1946]. Soon, the pharmacists Louis Goodman and Alfred Gilman, recruited by the US Department of Defense to investigate potential therapeutic applications of chemical warfare agents, observed that exposure to mustard gas caused profound lymphoid and myeloid suppression suggesting its utility for the treatment of lymphomata [Goodman et al. 1946]. Sidney Farber of Boston recognized that folic acid stimulated the proliferation of leukemia cells. In one of the first examples of rational drug design, he collaborated with Lederle Laboratories to devise folate analogs. He demonstrated that aminopterin produced remission in acute leukemia in children because it blocked a critical chemical reaction needed for DNA reduplication [Farber et al. 1948]. Aminopterin was the predecessor of methotrexate (developed by Lederle Laboratories in 1948), which in 1956 became the first compound cure of metastatic cancer, when it was used by Roy Hertz and Min Chiu Li to treat a case of choriocarcinoma. From 1942, research by George Hitchings and Gertrude Ellion at the Burroughs Wellcome Corporation had corroborated that it was possible to treat cancer with chemical compounds. Using one of them, 6-mercaptopurine, Joseph N. Burchenal (1912–2006) achieved a high percentage of complete remissions in childhood leukemias. Due to these early successes, the US Congress created a National Cancer Chemotherapy Service Center (NCCSC) at the National Cancer Institute in 1955. In 1965, cisplatin was discovered by Barnett Rosenberg, who explored the effects of electric fields on the growth of bacteria. He observed that the bacteria unexpectedly ceased to divide due to the exposure to an electrolysis product of the platinum electrodes. The discovery soon initiated studies into the effects of platinum compounds on cell division. This drug was later pivotal in the cure of testicular cancer. The often adverse effects of these agents were diminished when it was realized that they could be effectively used in combination [Frei et al. 1958; Frei et al. 1965]. This approach followed the strategy of antibiotic therapy for tuberculosis, which used combinations of drugs with different mechanisms of action. Frei, Freireich, and Holland hypothesized that cancer cells would be less likely to mutate and develop drug resistance to the drug combination (Table 1.3.C). The coalescence of efforts to eliminate compounds with intrinsic mutagenic potential from cancer therapy with increasing insights into the molecular pathways associated with growth signals led to the development of small molecule inhibitors, including STI571 (Gleevec) [Druker and Lydon 2000] and ZD1839 (Iressa).

Radiation carcinogenesis. In 1895, Wilhelm Conrad Röntgen (1845–1923), experimenting with electrical discharges in vacuum tubes (Crookes tubes), identified penetrating radiation that also produced

Table 1.3.C. Categories of conventional anticancer drugs. Chemotherapy is the use of chemical substances to treat cancer. The groups of classical anticancer agents comprises cytotoxic drugs that interfere with cell proliferation through various mechanisms

Alkylating agents: cross-link two DNA strands
Nitrogen mustards: Chlorambucil, Chlormethine, Cyclophosphamide, Ifosfamide, Melphalan
Nitrosoureas: Carmustine, Fotemustine, Lomustine, Streptozocin
Platinum: Carboplatin, Cisplatin, Oxaliplatin
Others: Busulfan, Dacarbazine, Mechlorethamine, Procarbazine, Temozolomide, ThioTEPA, Uramustine
Anti-metabolites: have affinity to enzymes of nucleic acid biosynthesis, "false building blocks"
Folic acid: Methotrexate, Pemetrexed, Raltitrexed
Purine: Cladribine, Clofarabine, Fludarabine, Mercaptopurine, Tioguanine. Pyrimidine: Capecitabine
Others: Cytarabine, Fluorouracil, Gemcitabine
Antibiotics: generate free radicals through redox cycles
Anthracyclines: Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Mitoxantrone, Valrubicin
Others: Bleomycin, Hydroxyurea, Mitomycin
Alkaloids: inhibitors of mitosis
Taxanes: Docetaxel, Paclitaxel
Vinca alkaloids: Vinblastine, Vincristine, Vindesine, Vinorelbine
Topoisomerase inhibitors: interference with DNA transcription and replication Type 1: Topotecan, Irinotecan
Type 2: Etoposide, Teniposide

fluorescence, and named it X-rays ("X" symbolizing the unknown). He died from leukemia after years of working with these newly discovered rays. In 1896, Henri Becquerel observed that penetrating radiation was given off by uranium. Marie Curie (born Maria Sklodowska, 1867–1934) discovered the element radium, as well as methods for separating radium from radioactive residues in sufficient quantities to analyze its therapeutic properties. After a life time of research into radioactivity, Marie Curie succumbed to pre-leukemia. The hazards of exposure to ionizing radiation were soon recognized. Acute skin reactions were observed in many individuals working with the recently invented X-ray generators. In the early years of the 20th century, these researchers were frequently affected by skin cancers and leukemias. By 1902, a case of radiation-induced cancer was reported, arising in an ulcerated area of the skin. Within a few years, a large number of such skin cancers had been observed, and the first report of leukemia in five radiation workers appeared in 1911. The French physician Jean Bergonie developed the law of radiosensitivity. He died in 1925 from cancer caused by his research with X-rays. In 1927, Hermann J. Müller recognized that ionizing radiation, already known to be carcinogenic, is also mutagenic [Müller 1927]. X-rays break the sugar–phosphate backbone of DNA. Radiation damage may be exerted by directly and indirectly ionizing radiation. Photons and neutrons are not charged and are indirectly ionizing. Radiation of charged particles (α-rays, electron rays including β-rays, proton rays) bear a higher risk for cellular damage, including transforming events. The atomic bombs that exploded over Hiroshima and Nagasaki caused dramatic increases in the incidence of leukemias during the ensuing decades. By the 1950s, researchers at the Sloan-Kettering Institute in New York City became alarmed over thyroid cancers that were diagnosed in adolescents who had received radiation treatment of their thymus glands in childhood. Later reports began to document that thyroid cancers could develop about 20 years following childhood radiation therapy. Nevertheless, the use of radiation to fight cancer was under study early on. The work by Maude Menton (1879–1960), Simon Flexner, and J.V. Jobling at the Rockefeller Institute lead to the publication of the monograph "Influence of Radium Bromide on a Carcinomatous Tumor of the White Rat" in 1910.

Viral carcinogenesis. Tumor viruses were detected at the turn of the 20th century with the cell-free transmission of human warts [Ciuffo 1907] and of chicken leukemia [Ellermann and Bang 1908]. In 1911, Peyton Rous isolated a highly oncogenic retrovirus (Rous sarcoma virus) from a chicken sarcoma [Rous 1911]. In 1932, Shope and Hurst demonstrated that papillomavirus had oncogenic activity in rabbits. In the early 1940s, Clarence Cook Little argued that viruses had caused breast cancer in a strain of laboratory mice. These groundbreaking results had been met with skepticism, because transmissibility in chickens and tumorigenesis in rabbits were not seen as applicable to human disease. The doubts were dispelled in the 1950s, by the demonstration that a tumor induced by Rous sarcoma virus (RSV) could produce infected tumor cells [Rubin 1955]. In conjunction with the observation that murine leukemia viruses are transmissible to newborn animals [Gross 1950], it initiated two decades of intense research into animal viruses, including many retroviruses with tumorigenic properties in animals. In 1964, the Epstein-Barr virus (EBV) was observed by electron microscopy in cultured cells from Burkitt lymphoma [Epstein et al. 1964]. Studies of RSV lead to the identification of the first oncogene, *v-src*, in the 1970s [Martin 1970; Brugge and Erikson 1977] and its subsequent sequencing [Czernilofsky et al. 1980]. In general, the infection of cells with an oncogenic DNA virus may result either in productive lytic infection with cell death and the release of newly formed virus particles or in cell transformation to the neoplastic state with little or no virus production, but with the integration of viral genetic information into the cell DNA. The viral genes capable of causing transformation (viral oncogenes) typically belong to the latent group of genes, which allow the infected cells to stay alive. The viral oncogenes are then present in all of the resulting cancer cells. Transforming retroviruses carry oncogenes derived from cellular genes, that are involved in mitogenic signaling and growth control. Viral transforming genes are collectively called *v-onc*, and their normal cellular counterparts are collectively referred to as *c-onc*. DNA tumor viruses encode oncogenes of viral origin that are essential for viral replication and cell transformation. The long delay between infection and the occurrence of tumors suggested that viruses can act in tumor initiation, and that additional damaging influences are required for tumor promotion. It is estimated that

15% of all human tumors worldwide are caused by viruses. (Table 1.3.D)

Oncogenic DNA Viruses. Three major families of DNA viruses, including herpes viruses, hepadna viruses, and papilloma viruses, have oncogenic potential. Although the polyoma virus SV40 and adenoviruses induce tumors in some animal species they are not known to be causative for any human tumors.

The genomes of herpes viruses are double-stranded linear DNA molecules with sizes in the range of 140–170 kb. The initiation of transformation by oncogenic herpes viruses appears to depend on specific genes, although no single T antigens (tumor antigens) have been identified. EBV was discovered in 1964 by Epstein, Achong, and Barr in a biopsy from Burkitt lymphoma. It is a γ-1 herpes virus infecting all human populations, with a prevalence of over 90% in adults. Infection results in the establishment of a lifelong carrier state, characterized by the persistence of antibodies to several viral gene products and the secretion of infectious virus in the saliva, which is also the usual vehicle of transmission. The Epstein-Barr Virus, which is the agent of infectious mononucleosis, is causative for Burkitt lymphoma (described by English surgeon Denis Burkitt in Uganda in 1958) in Africa and sporadic cases elsewhere, for B-cell lymphomata in acquired immunodeficiency syndrome (AIDS), as well as for nasopharyngeal carcinoma with high prevalence in China. Viral DNA and various EBV antigens are detectable in the affected tumor cells. A herpes virus designated HHV type 8 (KSHV, Kaposi sarcoma-related herpes virus) has been implicated in AIDS associated Kaposi sarcoma [Chang et al. 1994], the most common malignant tumor in AIDS, and also in rare sporadic Kaposi sarcomata unrelated to AIDS. The herpes simplex virus type 2 (HSV-2) may be involved in the pathogenesis of cervical cancer.

Table 1.3.D. Tumor viruses. Viruses can cause transformation. Tumor viruses belong to various taxonomic families. Like chemical carcinogens, they typically display organ selectivity

Virus	Cancer	Size of genome (kb)
DNA tumor viruses		
Herpes viridae		100–200
Epstein-Barr virus	Burkitt lymphoma, B-cell lymphoma, nasopharyngeal carcinoma	172
Human herpesvirus 8	Kaposi sarcoma	165
Hepadna viridae		
Hepatitits B virus	Liver cancer	3
Papova viridae		
Papillomavirus	Cervical carcinoma	8
SV40	Mesothelioma	5
Polyomavirus		
Adeno viridae		35
RNA tumor viruses		3–9
Oncorna viridae		
HTLV-1	T-cell lymphoma	9
HTLV-2	Hairy T-cell leukemia	
Flavi viridae		
Hepatitis C virus	Liver cancer	10

Originally known as serum hepatitis, hepatitis B has only been recognized as such since 1947. It has caused epidemics in parts of Asia and Africa. Hepatitis B is recognized as endemic in China and various other parts of Asia. Hepatitis B viruses (HBV) specifically infect liver cells. Chronic infection with HBV may have a causal role in primary hepatocellular carcinoma, which is one of the most common forms of cancer in Asia. Viral DNA is integrated into the tumor cells in some of these cases. In 1963, Baruch Blumberg and Harvey Alter reported the discovery of the hepatitis B surface antigen (Aa, HBsAg, Australia antigen), and a specific antibody binding to it. In 1970, Dane visualized the hepatitis B virion. These discoveries paved the way for the development of a vaccine.

The genomes of the papova family members polyomavirus and SV40 are double-stranded circular DNA molecules with sizes of about 5 kb. They contain two main groups of genes that are associated with early and late events in the replication cycle. The early genes are transcribed soon after infection of a cell and their encoded proteins participate in viral DNA synthesis but are not structural components of the virions. The late genes encode proteins of the viral coat and capsid. In productive lytic infection, early proteins are formed transiently before the structural proteins are assembled into viral particles. When stable transformation takes place, viral DNA is integrated into the cellular chromosomal DNA and some of the early proteins are persistently synthesized, but viral particles are not produced. Approximately 60–120 distinct types of human papilloma viruses (HPV) have been identified, which infect epithelial cells. While several forms cause benign tumors, such as warts, some types of sexually transmitted HPV are associated

with precursor lesions to squamous carcinoma of the uterine cervix. In 1983, Harald zur Hausen and colleagues isolated HPV16 from a human cervical cancer specimen. HPV types 16 and 18 ("high risk HPV"), followed by HPV types 45 and 31, may cause invasive cervical carcinoma or anorectal cancers. HPV DNA is extrachromosomal in the precursor lesions and infectious virus is produced. Viral DNA is frequently integrated into the cancer cells, but additional agents or factors may be involved at various stages of the progression to invasive carcinoma. Cell transformation by HPV results from the expression of two early genes, *e6* and *e7*. *e6* binds to P53, while *e7* binds to RB, in both cases resulting in the degradation of their targets in the Ubiquitin-proteasome pathway. Acting together, *e6* and *e7* are sufficient to induce transformation in the absence of mutations in cell regulatory proteins. In 2006, a vaccine against high risk HPV strains came on the market.

While there is no evidence that SV40 can induce human tumors or that SV40 DNA is present in human tumor cells, it has been a valuable model in cancer research. The early proteins found in tumors induced by polyomavirus and SV40 are termed T (tumor) antigens. Polyomavirus produces large, middle, and small T antigens, of which the middle T antigen (55 kD) is necessary for transformation. This early protein is bound to the plasma membrane of transformed cells and activates signal transduction pathways that promote cell cycle progression. The two early proteins, T (large T, 94 kD) and t (small t, 17 kD), are formed from the same reading frame by alternative splicing. The large T antigen is located in the nucleus of infected cells and maintains the transformed state. Distinct domains of large T bind to P53 and RB, inhibiting their function. Because large T inhibits both proteins, expression of only the SV40 large T protein is sufficient to induce the transformation of certain cells.

Most adenoviruses only cause acute upper respiratory tract infections. Adenoviruses were discovered in adenomatous tissue in 1953 by Rowe. Their genomes are double-stranded linear DNA molecules with sizes of about 35–40 kb. In cells transformed by oncogenic adenoviruses, a region of the genome encoding early gene products, including the E1A and E1B oncoproteins, is transcribed. These transforming proteins inactivate the RB and P53 tumor suppressors, with E1A binding to RB and E1B binding to P53.

Oncogenic RNA Viruses. Hübner and Todaro postulated the existence of retroviral oncogenes [Hübner and Todaro 1969]. Among the many families of RNA viruses, only members of the retrovirus and flavivirus families are capable of transforming cells and inducing tumors. The genomes of retroviruses are single-stranded RNA molecules with a size range of 3–9 kb. All retroviruses contain a Reverse Transcriptase [Baltimore 1970; Temin and Mizutani 1970], and their reduplication requires the synthesis of a double-stranded DNA intermediate of the RNA genome. Some of the virally determined DNA becomes integrated into the host DNA as a provirus. Typically, there are three retroviral genes that encode proteins necessary for viral reduplication, but do not contribute to transformation:

– The *gag* gene encodes internal structural proteins of the virus.
– The *pol* gene encodes Reverse Transcriptase.
– The *env* gene encodes envelope proteins that enclose the virus particles and largely determine the host range.

Most oncogenic retroviruses also possess one, or rarely two, oncogenes, termed *v-onc*. Under the influence of the viral promoter sequence, the *v-onc* gene is transcribed along with other viral genes and is responsible for the neoplastic transformation of the infected cell. Some of them promote growth, while others inhibit programmed cell death. More than 20 such oncogenes have been isolated and characterized. They include:

– In the class of growth factors: *v-sis* (Simian sarcoma virus)
– In the class of receptor protein Tyrosine Kinases: *v-erbA* (avian erythroblastosis virus), *v-erbB* (avian erythroblastosis virus), *v-fms* (feline sarcoma virus), *v-kit*
– In the class of nonreceptor Tyrosine Kinases: *v-abl* (Abelson leukemia virus), *v-fes*, *v-fps*, *v-src* (Rous sarcoma virus)
– In the class of serine/threonine protein kinases: *v-mil*, *v-mos*, *v-akt*, *v-raf*
– In the class of G-Proteins: *v-H-ras* (rat sarcoma virus, Harvey strain), *v-K-ras* (rat sarcoma virus, Kirsten strain)
– In the class of transcription factors: *v-ets*, *v-fos*, *v-jun* (avian sarcoma virus), *v-myc* (avian myelocytomatosis virus), *v-myb* (avian myeloblastosis virus), *v-rel*
– In the class of inhibitors of apoptosis: *v-flip*, *v-bcl-2*

Two unique types of human retroviruses, human T-cell leukemia viruses (HTLV) types 1 and 2 take part in the etiology of leukemias [Ruscetti et al. 1977; Mier and Gallo 1980; Poiesz et al. 1980]. Human T-cell leukemia virus type 1 (HTLV-1), the first human retrovirus to be isolated and characterized, may be the causative agent of a relatively rare form of T-cell lymphoma that occurs mainly in Japan and the Caribbean Islands. HTLV-2 can cause hairy T-cell leukemia [Kalyanaraman et al. 1982]. All the known RNA-containing tumor viruses are classified as retroviruses, with the exception of the hepatitis C virus (HCV), which resembles a flavivirus. In 1989, Daniel Bradley provided Chiron with non-A/non-B hepatitis serum from chimpanzees. There, Michael Houghton and colleagues discovered a single virus and changed the name to HCV. The virus was then cloned from infectious sera of patients with posttransfusion hepatitis. Hepatitis C may lead to chronic liver disease and cirrhosis, which is a predisposing factor for liver cancer.

1.4 SOMATIC THEORIES

The encounter with a family, in which many members developed breast or liver cancer, led Pierre Paul Broca to hypothesize, in 1866, that an inherited abnormality within the affected tissue caused the tumor development [Broca 1866]. From 1895 through 1913, Warthin studied the pedigrees of cancer patients at the University of Michigan Hospital. He identified four multigenerational families with susceptibilities to specific cancer types that appeared to be transmitted as autosomal dominant Mendelian traits [Warthin 1913]. These observations were put on mechanistic footing by 1900, when Hugo de Vries, Carl Correns, and Erich von Tschermak rediscovered the laws of inheritance, previously formulated in 1865 by Gregor Mendel (1822–1884). The chromosomes had been discovered by Walther Flemming (1843–1905) in 1877. He had described cell division and in 1882 coined the term "mitosis". In 1890, David von Hansemann had advanced the hypothesis that irregularities of the mitotic process are responsible for disordered growth [von Hansemann 1890]. Theodor Boveri (1862–1915) then proposed that defects in chromosomes lead to malignancy [Boveri 1914]. He hypothesized that malignant tumors might be the result of a certain abnormal condition of the chromosomes, which may arise from multipolar mitosis. The main concepts of Boveri's theory are:

– The problem of tumors is a cellular problem
– Typically, every tumor arises form a single cell
– The primordial cells of tumors contain, as a result of an abnormal process, definite and wrongly combined chromatin contents
– Chromosome abnormalities are the cause to the tendency toward rapid cell proliferation, which is passed on to all decendents of the primordial cell.

In the 1950s, Sajiro Makino in Japan, Theodore Hauschka in the United States, and Albert Levan in Sweden observed that virtually all tumor cell lines have chromosomal aberrations. The discovery of the Philadelphia chromosome in chronic myeloid leukemia [Nowell and Hungerford 1960] later provided experimental evidence for Boveri's theories. It supported the hypothesis that damage to the chromosomes induced carcinogenesis. Aneuploidy, typically with elevated DNA content, is a frequent marker of cancerous cells. Providing more functional insight, the first description of a translocation was reported in 1973 by Janet D. Rowley [Rowley 1973]. Although the Philadelphia chromosome was among the first translocations to be discovered, the genes involved in the translocation that causes Burkitt lymphoma were the first to be molecularly characterized. In 1982, Carlo Croce and Bob Gallo showed that the *myc* proto-oncogene on chromosome 8 is affected by the translocation. Simultaneously, Phil Leder's group demonstrated that *myc* is translocated into the 5′ region of the *immunoglobulin heavy chain* (*igH*) gene [Dalla-Favera et al. 1982; Taub et al. 1982].

Cancers represent a large category of somatic cell genetic diseases [McKusick 1985]. The term "somatic mutation" was first applied to cancer by Ernest Tyzzer, who observed that tumors sequentially transplanted into mice developed a continuous broadening of host specificity among recipients from various inbred strains [Tyzzer 1916]. By the 1970s, Tyzzer's model had received a molecular underpinning and cancer was understood as a disease of genetic alterations. Tumor initiation and progression occurs through the accumulation of changes that begin when a single normal cell sustains a permanent genetic damage. The resulting dysregulation of gene function is responsible for the clonal expansion of a population of somatic cells that ultimately becomes dominant.

Progress in the understanding of DNA and genes has been a major determining factor for progress in cancer research. In 1869, Johann Friedrich Miescher had identified a weakly acidic substance of unknown function in the nuclei of human white blood cells, which later became known as deoxyribonucleic acid, or DNA. The term gene (derived from the Greek γενοσ = origin), attributed to Johanssen, first appeared in 1909 as an abstract concept to explain the hereditary basis of traits. Oswald Avery, Colin McLeod, and Maclyn McCarthy showed in 1944 that DNA constitutes the genetic material. In 1953, James Watson and Francis Crick deduced the double helical structure of DNA from X-ray diffraction data, generated by Rosalind Franklin. In 1961, Sidney Brenner and Francis Crick established that groups of three nucleotide bases, or codons, are used to specify individual amino acids. The genetic code of nucleotide triplets was worked out in final detail in 1966, mainly through work by Marshall Nierenberg and Heinrich Matthaei. This paved the way for the molecular analysis of gene damage.

One of the most important approaches for biotechnology is the cloning of genes inserted into plasmids. It was initiated through discussions between Stanley Cohen and Herb Boyer at a conference in Hawaii, and by March 1973 the feasibility of their new method was demonstrated. PCR was invented by Kary B. Mullis in spring of 1983. These techniques allowed for the large availability and easy manipulation of cancer related genes. In 1977, Frederick Sanger at the Medical Research Council in Cambridge, UK and Walter Gilbert at Harvard University in Boston, USA independently devised methods for sequencing DNA, which were further developed by Leroy Hood at the California Institute of Technology, who invented an automated DNA sequencer in 1985. In 1990, the Human Genome Project was launched to obtain the complete blueprint of human DNA, planned for 2005. In 1998, the competition by a private enterprise, led by Craig Venter, accelerated the process, so that both groups presented a draft sequence of the genome by June 2000. The genetic analysis of cancer experienced additional support from the technical accomplishment to manipulate individual genes in vivo. In 1982, a team led by Richard Palmiter and Ralph Brinster generated the first transgenic mouse. This was achieved through pronuclear microinjection of genetic material into the nuclei of fertilized eggs. From 1987 through 1989, teams led by Martin Evans, Oliver Smithies, and Mario Capecchi created knockout mice by selectively disabling a specific target gene in embryonic stem cells.

RNA tumor viruses can cause normal cells to adopt the characteristics of rapid uncontrolled growth that are typical of many tumors. The discovery of the human proto-oncogene *src* by Dominique Stéhelin, Harold Varmus, Michael Bishop, and Peter Vogt [Stéhelin et al. 1976] confirmed that viral oncogenes are derived from related genes of host cells. Their analysis implied that the cellular *src* sequence is involved in the normal regulation of growth. It also suggested that tumors could arise independently of viruses as a result of mutations in their related cellular genes. Consecutively in 1982, three publications in the journal Nature independently of one another identified a point mutation in the proto-oncogene *ras* as a defect associated with bladder cancer [Chang et al. 1982; Parada et al. 1982; McBride et al. 1982]. These discoveries revealed that a cellular transforming gene involved in human bladder and lung tumors was homologous to the transforming viral *ras* gene [Parada et al. 1982; Der et al. 1982], and that an activating point mutation affected the identical codon in all cases. Thus, it became apparent that the same cellular proto-oncogenes could be affected by viruses, by chemical carcinogens, or by nonviral somatic mutations, which brought together various previously independent lines of research.

The observation that the growth of murine tumor cells in vivo could be suppressed by fusion of the tumor cells with nontransformed cells provided evidence that the ability of cells to form a tumor is a recessive trait [Ephrussi et al. 1969]. Knudson [Knudson 1971] carried out an epidemiological study of retinoblastoma development in children. He postulated that "two hits" are required for the complete inactivation of a tumor suppressor gene. The gene *p53* was discovered independently by Linzer and Levine [1979] and by Lane and Crawford [1979] as a cellular protein that binds to the viral oncoprotein of SV40. Initially suspected as a cellular oncogene, due to mutations that act as dominant negative forms, the identification of loss of heterozygozity and loss of function mutations of *p53* confirmed its actual role as a tumor suppressor [Baker et al. 1990]. After this clarification, P53 became known as the guardian of the genome,

because it protects from the consequences of genetic damage by inhibiting cell division or inducing cell death. In 1983, loss of heterozygosity analysis was used to map the tumor suppressor gene *rb*, which was then cloned in 1986 [Friend et al. 1986].

Oxidative metabolism inevitably leads to DNA damage. This may occur by direct oxidation of bases, by induction of DNA strand breaks, or by mediation of frameshift mutations in microsatellite DNA. Each cell (of estimated 10^{14} in the human body) loses more than 10^4 bases (out of a total of 6×10^9 nucleotides) per day from the spontaneous breakdown of DNA at body temperature, mostly through the damage by reactive oxygen species. A similar number of lesions is generated by spontaneous depurination, resulting in miscoding by the residual apurinic site [Loeb 2001]. The deamination of 5-methylcytosine to thymine is among the most frequent causes for point mutations. It accounts for more than 20% of all base mutations that give rise to genetic disease [Krawczak et al. 1998]. It has been estimated that 5-methylcytosine deaminates at a rate of 5.8×10^{-17} s^{-1} at each CpG site (cytosine and guanine separated by a phosphate) [Shen et al. 1994], which corresponds to about four residues per cell per day. Mutation frequencies of the *hypoxanthine phosphoribosyl transferase* (*hprt*) gene, a commonly used marker for mutation frequency, in normal adult epithelial cells reach approximately 1.3×10^{-4} [Martin et al. 1996].

The reduplication of DNA during cell division introduces the possibility of errors at an estimated rate of 1.4×10^{-10} nucleotides/cell/division. Loeb and colleagues [Loeb et al. 1974] realized that it would be unlikely for tumor cells to acquire the number of mutations presumably needed for full transformation during the lifetime of the host and postulated the existence of mutator genes. Much later, the study of hereditary non-polyposis coli led to the discovery of defective DNA repair genes [Ionov et al. 1993; Thibodeau et al. 1993; Parsons et al. 1993]. Any mutation of cancer associated genes can be handed on to following generations and predispose the affected cells to malignant transformation in the case of additional DNA damage. The formation of cancer has been termed "clonal evolution" to describe how certain mutations enable cells to copy their damaged DNA and divide under conditions, which cause normal cells to stop replicating. The repetition of this process allows cells to accumulate cancerous mutations [Cavenee and White 1995].

In 1949, Berenblum and Shubik [1949] concluded that carcinogenesis is at least a two-stage process. Five years later, Armitage and Doll [1954] inferred from their analysis of age and cancer incidence a 6–7 step process. In 1983, Newbold and Overell observed that an activated *ras* gene failed to transform normal fibroblasts, unless they were first immortalized [Newbold and Overell 1983]. This led to the hypothesis that *ras* activation was only one step in a number of mutations necessary in the pathway to malignancy. The concept of multiple somatic mutations as underlying mechanism of carcinogenesis was further advanced by a multistep carcinogenesis model, conceived of by Foulds [1957] and refined by Fearon and Vogelstein [1990]. It also gave rise to the recognition of chromosome instability and microsatellite instability as two distinct pathogenetic mechanisms of carcinogenesis. The technical achievements of differential display [Liang and Pardee 1992; Liang et al. 1992], serial analysis of gene expression (SAGE) [Velculescu et al. 1995; Zhang et al. 1997], and DNA microarrays [Schena et al. 1995; DeRisi et al. 1996] further advanced these concepts to the definition of transformation on the basis of aberrant gene expression profiles [Kononen et al. 1998; Golub et al. 1999].

In addition to chromosome integrity and DNA sequence fidelity, the regulation of the chromatin structure is an important determinant in transformation. DNA methylation is a covalent modification of the C5 position in cytosine. This methylation pattern is stably maintained at CpG dinucleotides by a family of DNA Methyl Transferases that recognize hemi-methylated CpG dinucleotides after DNA replication. DNA hypo-methylation was identified as a characteristic of cancer cells in 1983 [Feinberg and Vogelstein 1983]. In 1964, Vincent Allfrey had realized that Histones were often chemically modified by acetylation, which caused them to relax their binding to DNA [Allfrey et al. 1964]. This implied the possibility of a role for histones in cancer [Roth 1965]. In 1974, Robert Kornberg proposed that chromatin was quite structured, consisting of repeated units of about 200 base pairs of DNA wrapped around 2–4 distinct Histones (later called nucleosomes) [Kornberg 1974; Kornberg and Thomas 1974]. The importance of acetylation for

the regulation of gene expression and gene silencing was, however, realized only many years later. In 1998, methylation and phosphorylation of Histones were observed by several investigators to contribute similarly [Bestor 1998]. Today, various enzymes that modify Histones are known to contribute to transformation [Horiuchi et al. 1981].

1.5 REGULATORY THEORIES

Beyond the development of cancer research from explanations on a cellular level to a molecular genetic level, there has been a development of dynamic models of carcinogenesis. Winge introduced the concept of selective cellular proliferation, realizing that selection must operate on a genotypically mixed population of proliferating cells as inevitably as it acts on a genotypically mixed population of reproducing organisms [Winge 1930]. Macfarlane Burnet conceptualized the clonal selection theory for immunity and applied it to cancer. It suggests that tumorigenesis represents the development of a clone of cells with the capacity to multiply excessively in the context of its relationships within the body [Burnet 1959]. In the 1960s, feedback control in biological systems was described by Francois Jacob and Jacque Monod. Cellular metabolism and proliferation are regulated by spatiotemporal circuits of mutual feedback control. They include extracellular and intracellular signals, rate limiting steps, and checkpoint controls. Cancer development has also been described with the algorithms of ecology [Michelson et al. 1987; Maley et al. 2006] and game theory [Tomlinson 1997]. The regulatory theory contends that cancer is not a morphologic entity, but an aberrant regulatory process among individual cells, their microenvironment, and the entire host. Genetically identical cells and organisms exhibit substantial diversity, even when they have identical histories of environmental exposure. Variation in gene expression, based in part on the stochastic nature of biochemical reactions, may contribute to this phenotypic variability [Raser and O'Shea 2005]. Genetic changes underlying growth control, senescence, invasion, and stromal–parenchymal interactions are part of a continuum of carcinogenesis that affects interrelated pathways. In malignant cells, the normal balance between the number of cells completing the cell cycle and the number of cells dying is changed. Likewise, the balance of adhesive versus migratory surface molecules on malignant cells is shifted in favor of the motility enhancing receptors.

Full transformation has two basic requirements:

– Genetic instability of the cell to drive tumor progression
– Selective advantage of the cell to allow for clonal expansion [Cairns 1975; Nowell 1976].

The genetic instability of tumor cells is reflected in the heterogeneity within individual tumors and among tumors of the same type. It is based either on chromosome instability, leading to aneuploidy, or on defective DNA repair, leading to microsatellite instability and gene mutations. Genomic destabilization is an early event in tumor development. The mean number of alterations in a cell that turns carcinomatous may amount to about 11,000 [Stoler et al. 1999]. Waves of clonal expansion give rise to daughter cells that have the growth advantage typical of cancer. Clonal selection drives this process. Tumors are clonal insofar as they are derived from the same stem cell precursor. Genetic instability generates a collection of coexisting subclones, each with the potential for future changes in the face of selective pressures [Cahill et al. 1999]. The relative importance of selective advantage versus genetic instability in tumor initiation and progression is still subject to debate.

Studies of cell senescence have led to a research focus on population dynamics, selection, and evolution. Hayflick recognized that there is a finite number of possible population doublings by nontransformed differentiated cells [Hayflick and Moorehead 1961]. After a limited number of divisions, a state of crisis is reached, in which most cells die. A few cells may be altered in a fashion that conveys a selective advantage, which allows them to grow out and dominate the population. These cells are selected and form an expanding population with potentially precancerous characteristics. The demonstration that HTLV-1 immortalizes normal T-lymphocytes [Popovic et al. 1983] led to additional investigations, which confirmed that tumor viruses can frequently immortalize human host cells. The shortening of the chromosome ends, telomeres [Szostak and Blackburn 1982; Moyzis et al. 1988], is an integral part of replicative senescence. The enzyme Telomerase [McKay and Cooke 1992; Chong et al. 1995] replenishes the chromosome ends and can prevent this shortening. Its activity is present in most cancer cells, but not

typically in nontransformed differentiated cells [Hastie et al. 1990].

The first cancer hospital was founded in the 18th century in Reims, France. French gynecologist Joseph Claude Anthelme Récamier (1774–1852) described the invasion of the bloodstream by cancer cells, coining the word "metastasis." In the 1850s, Pierre Paul Broca (1824–1880) and Karl von Rokitansky (1804–1878), independently of each other, observed the venous spread of cancer. Theories of metastasis formation have traditionally been based on concepts of population dynamics. In 1889, English surgeon Stephen Paget (1855–1926) described the propensity of various types of cancer to form metastases in specific organs. He stated that "the distribution of the secondary growth is not a matter of chance" and proposed that these patterns were due to the dependence of the "seeds" (the cancer cells) on the "congenial soil" (the target organ for metastasis) [Paget 1889]. This notion was challenged by American pathologist James Ewing (1866–1943), who suggested that circulatory patterns between a primary tumor and specific secondary organs were sufficient to account for most of the targeted metastasis [Ewing 1928]. This was relativized by Leonard Weiss, who demonstrated that the number of metastases in specific target organs, derived from certain tumors, could not be accounted for solely by blood flow patterns [Weiss 1992]. The first evidence that metastasis formation depends on intrinsic characteristics of the tumor cells came from experiments by Isaiah Fidler [Fidler 1975], who generated sublines with increasing invasive potential by serial passage of a melanoma cell line through mice. Soon, somatic cell fusion and microcell mediated chromosomal transfer suggested that the ability to disseminate was under positive and negative genetic control [Ramshaw et al. 1983; Sidebottom and Clark 1983; Layton and Franks 1986]. These observations placed ensuing research activities into metastasis on a deterministic footing. The secretion of proteases by tumor cells [Turpeenniemi-Hujanen et al. 1985; Matrisian et al. 1986] was recognized as one factor causing invasiveness. Homing receptors were identified on the cell surface, which are necessary and sufficient to mediate metastasis formation by specific tumors [Günthert et al. 1991]. In conjunction with the finding of metastasis suppressor genes [Steeg et al. 1988; Alvarez et al. 1990], the detection of metastasis genes has corroborated the existence of genetic programs intrinsic in the tumor cells, which regulate invasiveness. These observations have led to the development of a genetic theory of metastasis formation, according to which metastasis genes are developmentally nonessential genes that physiologically contribute to inflammation, wound healing, and stress-induced angiogenesis. Their dysregulation in cancer occurs on the level of aberrant expression and splicing [Weber and Ashkar 2000]. Tissue-specific molecular markers (Addressins) were identified in 1988 [Streeter et al. 1988], which implied the possibility that circulating cells could recognize target organs. This was corroborated by the identification of the contribution by Chemokines and their cognate receptors to tumor dissemination [Mueller et al. 2001].

In the evolution of research progress from a reductionist to a comprehensive understanding of cancer, interactions between the host and the cancer cells have recently received increasing attention. Mintz and Illmensee [1975] had demonstrated that the injection of undifferentiated embryonal carcinoma cells into mouse blastocysts suppressed their inherent tumorigenicity and led to the contribution by these cells to a variety of functional tissues. Around the same time, the Michigan radiologist John Wolfe recognized that women with dense breasts had an elevated risk of contracting breast cancer, implying a role for the stromal architecture. In 1990, it was realized that the tissue environment had a dramatic effect on the potential by tumors to metastasize [Nakajima et al. 1990]. Tumorigenic prostatic stroma and nontumorigenic prostatic epithelium can interact to induce the development of carcinosarcoma [Chung et al. 1988]. The concept that the stroma plays important roles in carcinogenesis has since been developed by Mina Bissell [Bissell and Radisky 2001], Judy Campisi [Krtolica et al. 2001], and Donald Ingber [Huang and Ingber 1999].

Early work in experimental carcinogenesis had shown vascularization and hyperemia around tumor transplants [Ide et al. 1939; Coman and Sheldon 1946] and similarities were seen between the vascular reactions to tumors and to tissue damage [Algire et al. 1945]. Cancer researchers became interested in angiogenesis factors in 1968, when the first hints emerged that tumors might release such substances to foster their own progression. Two groups, one led by Melvin Greenblatt in California with Phillipe Shubik in Chicago, and another by

Robert L. Ehrmann and Mogens Knoth in Boston, showed that burgeoning tumors can release a substance that induces existing blood vessels to grow into them [Rijhsinghani et al. 1968; Ehrmann and Knoth 1968]. Such vascularization promotes tumor growth because it ensures a sufficient supply of oxygen and nutrients. Folkman [1971; Folkman et al. 1971] recognized the important role of blood vessels in the growth of cancerous tumors. After more than a decade of research, mediators of angiogenesis that are secreted by some tumors were identified [Senger et al. 1983; Shing et al. 1984]. The inhibition of VEGF (Vascular Endothelial Growth Factor)-induced angiogenesis was shown to suppress tumor growth [Kim et al. 1993]. Today, a monoclonal antibody to VEGF is used in the treatments of some cancers. These investigations also led to the discovery of naturally secreted compounds that curtail the growth of new tumors [Taylor and Folkman 1982; O'Reilly et al. 1994; O'Reilly et al. 1997].

In the 17th and 18th centuries, some believed that cancer was contagious. In fact, the first cancer hospital in France was forced to move from the city in 1779 because of the fear that cancer could spread throughout the city. More than a century later, the potentially protective role of the immune system against transformed cells was recognized. In the 1890s, New York surgeon William B. Coley found a record of a young patient with round cell sarcoma on the neck, who had been listed as an utterly hopeless patient when he developed a severe infection of erysipelas. He survived the infection and his tumor went into remission. Based on this case, Coley devised a killed vaccine of *Streptococcus pyogenes* (the cause of erysipelas) with *Serratia marcescens*. After a few years of its use, he reported to have successfully treated some sarcoma patients with the application of his bacterial toxins (Coley's toxin) [Coley 1893, 1896]. After Coley's death, his daughter Helen Coley Nauts reviewed his records, published several reviews of his work, and founded the Cancer Research Institute, which promotes immune therapies for cancer. In 1909, Paul Ehrlich carried out immunizations in animals with tumor cells and suggested that tumors occur at high frequency in humans, but are kept under control by the immune system [Ehrlich 1909]. Further developments in tumor immunology have led to models of selection and evolution of cancer cells. Macfarlane Burnet coined the term immunosurveillance in 1967 [Burnet 1967]. In this conceptual framework, the host immune system constantly screens cells for signs of transformation and eliminates those that pose a threat to the body's integrity. The growth of a tumor reflects an escape from immunosurveillance. Cancer cells that can evade the immune system, be it by down-regulation of antigen presenting or co-stimulatory molecules, be it by expression of immunosuppressive cell surface molecules or cytokines, will grow out and form tumors. Three distinct theories were developed to interpret the nature of the tumor recognition by the immune system.

- Lewis Thomas described homograft rejection as a primary defense against neoplasia [Thomas 1959].
- According to concepts by Burnet, which are based on self/non-self discrimination, the immune system is active early in antitumor protection. The early surveillance mechanisms shape the tumor's immunological phenotype [Burnet 1967]. This was supported by the description of tumor specific antigens [Old and Boyse 1964]. Tumors mostly express self antigens, which may account for the incomplete protection from transformation by the immune system.
- The alternative proposal of the danger theory [Matzinger 1994] implies that the immune system is activated only at later stages of carcinogenesis. During the early stages, tumor cells appear immunologically as healthy growing cells that do not send out danger signals to activate the immune system because they express neither microbial immune recognition patterns nor release distress signals to alarm the innate immune system cells [Fuchs and Matzinger 1996]. In advanced growth, hypoxia and tissue damage induce stress responses, which activate the immune system. In the framework of the danger theory, the immune system is activated at later stages of tumor development, when tissue damage has occurred.

The possibility to direct the immune system to fight cancer cells in virtually any location within the body with minimal side effects has attracted increasing research efforts. The high specificity and high binding affinity of antibodies made them attractive as potential anticancer agents. For a long time, however, they were difficult to isolate in large quantities. The fusion of antibody producing cells with myeloma cells into hybridomas, accomplished by Cesar Milstein and Georges Koehler in the early 1970s, changed that. Yet, biotechnology had to advance to accomplish humanizing such antibodies before they became successful in therapy. In 1997,

the US Food and Drug Administration (FDA) approved Rituxan, a monoclonal antibody to CD20 (developed by IDEC Pharmaceuticals) to treat non-Hodgkin lymphoma [McLaughlin et al. 1998]. The process also led to the development of Herceptin, spearheaded by Dennis Slamon, an antibody that targets the receptor ERBB2 (HER-2/NEU) that is overexpressed on the surface of about 30% of breast cancers. Because antitumor immunity is predominantly cellular immunity, other research has been directed toward turning T-lymphocytes against tumors. Steven A. Rosenberg focused his efforts to generate antitumor vaccines on tumor associated antigens. In a similar approach, Martin Kast studied the development of peptide-based vaccines. Glenn Dranoff demonstrated the high effectiveness of irradiated tumor cells transfected with the cytokine GM-CSF in inducing antitumor immunity [Dranoff et al. 1993]. Over time it became clear, on the other hand, that the immune system could also impact negatively on cancer risk in the context of chronic inflammation. In 1876, Robert Koch and Louis Pasteur had shown independently of each other that microorganisms can cause disease. In the 1980s, Barry J. Marshall and J. Robin Warren demonstrated that gastric ulcers were caused by bacteria they called *Helicobacter pylori*. Infection results in widespread inflammation that predisposes to stomach cancer. Inflammation in the stomach mucosa is also a risk factor for MALT (mucosa-associated lymphoid tissue) lymphoma, a lymphatic neoplasm in the stomach.

Over the decades, the roles of hormones in carcinogenesis have received increasing attention. The observation by Bernardino Ramazzini in 1713 of a virtual absence of cervical cancer and relatively high incidence of breast cancer in nuns was an important step toward identifying and understanding the importance of hormonal factors, such as those associated with pregnancy, in modifying cancer risk. In 1878, Thomas Beatson discovered that the breasts of rabbits stopped producing milk after he removed the ovaries. He suggested to the Edinburgh Medico-Chirurgical Society in 1896: "This fact (. . .) pointed to one organ holding control over the secretion of another and separate organ." Beatson found that oophorectomy often resulted in the improvement of breast cancer patients and inferred the stimulating effect of a female ovarian hormone on breast cancer, before the hormone itself was discovered [Beatson 1896]. Allen and Doisy [1923] identified an ovarian hormone they referred to as "estrus stimulating principle," later called estrogen. From the late 1950s to the 1970s Elwood Jensen demonstrated that such hormones do not undergo redox modifications to become activated. Instead, they bind to a receptor protein within their target cells [Jensen and Jacobson 1962]. This hormone/receptor complex then travels to the cell nucleus, where it regulates gene expression. The first nonsteroidal antiestrogen to be reported in the literature, MER25, was described by Lerner and coworkers in 1958 [Lerner et al. 1958] as an agent that had no other hormonal or antihormonal properties. The drug failed in clinical trial because the large doses required caused serious central nervous system side effects. Tamoxifen, first discovered in 1962, is a nonsteroidal antiestrogen that serves a dual role as breast cancer treatment and preventive. It was approved for the treatment of advanced breast cancer by the US FDA in 1977. Awareness of the androgen dependence of prostate tissue can be traced back to the Scottish surgeon John Hunter, who observed in 1786 that castrated bulls had small prostates. In 1941, Charles Brenton Huggins (1901–1997), a urologist at the University of Chicago, with his students Clarence V. Hodges and William Wallace Scott, published three papers that demonstrated the relationship between the endocrine system and the normal functioning of the prostate gland. In the 1940s, Charles Huggins also reported a dramatic regression of metastatic prostate cancer following removal of the testes [Huggins and Hodges 1941]. Later, drugs that blocked male hormones were found to be effective treatments for prostate cancer. Androgen ablation with Gonadotropin Releasing Hormone agonists (GnRH-As) in prostate cancer patients was first reported in 1982 [Tolis et al. 1982]. In 1988, the Androgen receptor was cloned [Chang et al. 1988]. Iatrogenic causes for cancer predisposition were incriminated by a study published in 1971, which documented an association between clear-cell adenocarcinoma of the vagina and in utero exposure to diethylstilbestrol [Herbst et al. 1971] (Dodds and associates had characterized diethylstilbestrol as an extremely potent estrogen [Dodds et al. 1938]; it had been prescribed for close to 30 years to prevent certain complications of pregnancy and as a treatment for advanced breast cancer in postmenopausal women). In July 2002, the Women's Health Initiative study was stopped after more breast cancers and heart problems occurred

among women taking estrogen–progestin pills. In 2006, multiple clinical studies showed that breast cancer rates in the United States dropped in 2003, consecutive to a drastic reduction in the use of hormone replacement therapy. Some of the numbers came from the National Cancer Institute's surveillance database, which uses cancer registries around the country to project national incidence and death rates.

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