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Models of Neoplasia and Their Diagnostic Implications: A Historical Perspective

David M. Goldberg[1,3] and Eleftherios P. Diamandis[2]

In comparison with normal cells, cancer cells have an enhanced ability to trap both nitrogen and energy; an enhanced operation of the glycolytic and direct oxidative pathways, leading to accumulation of lactate and increased production of NADPH; and a greater content of lysosomal hydrolases. These changes represent a reprogramming of gene expression, which, at its most specific, is accompanied by the reappearance in the cell and ultimately in the body fluids of oncodevelopmental proteins not normally found in mature adult tissues. The most florid stage of this reprogramming leads to the metastatic phenotype, which confers upon the cancer cell the ability to stimulate angiogenesis, invade the bloodstream and lymphatic vessel, and arrest and proliferate in distant tissues. The diagnostic implications of these phenotypic changes are illustrated for cancer of the cervix uteri and cancer of the colon. We also review the classical theories of neoplasia, including the cellular anoxia concept of Warburg, the deletion hypothesis of Potter, and various other mechanisms emphasizing genomic derepression and impaired immunity. The critical steps in chemical carcinogenesis are described, and the Vogelstein–Lane model is presented, emphasizing the stepwise and cumulative genomic changes affecting chromosomes 5q, 17p, 18q, and gene amplification of chromosome 12 as well as genomic instability resulting from reduced DNA methylation. The main consequences of these genomic alterations include overexpression or activation of oncogenes such as c-*myc* and k-*ras*, together with mutation or functional inactivation of suppressor genes such as *p53*. Finally, the implications of these findings for diagnosis and management are illustrated by reference to recent investigations in cancers of the breast, colon, and bladder, in which these genomic alterations can be detected by examination of appropriate cellular material and by detection in serum of antibodies to the *p53* gene product.

Indexing Terms: *cancer · metastasis · lysosomal hydrolases · oncodevelopmental proteins · tumor markers · carcinogenesis · oncogenes · tumor-suppressor genes · acute-phase proteins*

The title of this review raises issues that can be fully addressed only by a textbook or treatise. Therefore, for practical purposes, we have had to very clearly define our scope and intentions. In brief, we describe the nature of the cancer cell, how its presence may be recognized, and how it comes to be what it is in the first place. These three objectives can be roughly translated into the following themes: the cancer phenotype; laboratory diagnosis; and mechanisms of carcinogenesis. Because these topics will form the basis of virtually every other presentation at this conference, we will not attempt to do more than to provide a background, which we hope will render these presentations more comprehensible and make it less necessary for succeeding authors to dwell on fundamental and well-recognized concepts.

Features of the Cancer Phenotype

If the genotype of cancer is complex and confusing, the phenotypic behavior and properties of cancer cells are much more so. Among the dilemmas that obfuscate any attempt at such a description is the fact that cancers comprise a mixture of many different cell types. Even those cells that are malignant may consist of several different clones, although one will almost always dominate a particular tumor. The behavior of cancer cells growing in culture may be very different from that which characterizes the same cells growing as a solid tumor. The properties of metastases may differ from those of the primary tumor because of the different environment in which the cells find themselves or because the metastases may arise from clonal expansion within the original tumor. Phenotypic modulation occurs in response to the activation of defense mechanisms, of which humoral and cell-mediated immunity are probably the most important. Finally, many of the features attributed to cancer cells, especially those that have traditionally been considered most useful in diagnosis, are the result of even more complex and generalizable tumor–host interactions mechanistically related to endocrine and nutritional functions (*1*). Table 1 lists some of these features. The following text will elaborate on the information provided in Table 1 and will also present a brief account of three further aspects of the cancer phenotype: reprogramming of gene expression, oncodevelopmental gene expression, and phenotypic features associated with metastatic potential.

Metabolic Features

Protein metabolism. Investigations in tumor-bearing animals and in human cancer patients suggest that a shift of nitrogen from normal to neoplastic tissues occurs as a consequence of the ability of cancer cells to "trap" nitrogen. Normal tissues become depleted, whereas the cancer cells are able to ensure a continuing supply of amino acids for protein synthesis and cell growth (*2–5*). Insofar as this is a valid phenotypic feature of cancer cells, it may be explained by enhanced activity of the

[1] Department of Clinical Biochemistry, University of Toronto, Banting Institute, 100 College St., Toronto, Ontario, M5G 1L5, and [2] Department of Clinical Biochemistry, The Toronto Hospital (Western Division), 399 Bathurst St., Toronto, Ontario, Canada M5T 2S8.

[3] Address correspondence to this author, at the Banting Institute. Fax 416-978-5650.

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Table 1. Features of the Cancer Phenotype

Protein metabolism
The nitrogen trap
Carbohydrate metabolism
The energy trap—hypoglycemia
Insulin resistance—hyperglycemia
Respiratory lesion—mitochondrial damage
1. Low tricarboxylic acid cycle activity
2. High glycolysis—lactic acidosis
3. High hexose monophosphate shunt activity—NADPH production
Lipid metabolism
The vanishing fat syndrome
Lysosomal hydrolases
The voracious appetite of the cancer cell
Preneoplastic changes
Histochemical markers—GGT
Oncodevelopmental gene expression
Tumor markers—CEA, α-fetoprotein, Regan isoenzyme, etc.

γ-glutamyl cycle. This has been proposed as a mechanism for the uptake of amino acids by cells (*6*). Believed to be relatively unimportant in most normal tissues, it could conceivably be a major device for the binding, translocation, and intracellular transfer of amino acids into cancer cells specifically, by virtue of the high content of the relevant enzymes such as γ-glutamyltransferase (GGT), which these cells are known to possess (see reference *7* for a review).

Carbohydrate metabolism. Hypoglycemia is a common feature of patients with advanced cancer. Energy balance studies have demonstrated that cancer cells are as successful in trapping energy as in the preferential capture of amino acids (*5*).

A feature widely but not invariably found in cancer cells is a block in the intracellular respiratory (electron transfer) mechanism (*8*). This is frequently accompanied by mitochondrial damage and a reduction in the tricarboxylic acid cycle activity. To compensate for this, two alternative pathways are exaggerated: glycolysis, which is often high even under aerobic conditions, leading to high rates of lactic acid production (*8, 9*) and a fall in the pH of the tumor tissue (*10*), and activity of the direct oxidative pathway, otherwise known as the hexose monophosphate shunt (*11–13*). The latter is important in the economy of the cancer cell by contributing in a major way to the production of NADPH, which is necessary for nucleic acid synthesis.

Lipid metabolism. A dramatic reduction in body fat content is invariably found in terminal cancer patients (*14*). This, together with the effects of prolonged negative nitrogen balance and protein wasting, leads to the clinical picture of cachexia. The factors that induce this state are multiple and complex and involve endocrine and nutritional mechanisms that operate at the level of the host rather than of the cancer cell (*15*).

Lysosomal hydrolases. An excess of lysosomes is often found in cancer cells (*16*). This confers a selective advantage, in that intracellular digestion is enhanced, and is likely to prove nutritionally beneficial to cancer cells. These enzymes probably also play an important role in the local spread of the tumor and in its ability to metastasize.

The Molecular Correlation Concept

This concept was proposed by George Weber on the basis of a monumental series of investigations summarized in his Clowes Memorial Lecture in 1983 (*17*). To this day, it stands as the most comprehensive characterization of the cancer cell in terms of its metabolic behavior. Comparing normal tissues (mainly liver) with a series of tumors of varying but defined growth rates (mainly hepatomas), he recognized that certain key enzymes are tightly coupled with either neoplastic transformation or with progression of the tumor by alterations in their activity, concentration, or isoenzyme profile. These key changes, shared in greater or lesser measure by all cancer cells, depending on their grade of malignancy, proliferative rate, and degree of differentiation, could be distinguished from coincidental changes in gene expression not strictly linked with neoplasia. An example of this approach is illustrated in Figure 1. The overall changes in gluconeogenic enzymes typical of cancers are summarized in Table 2.

Although Weber was not successful in explaining the mechanism leading to this reprogramming of gene expression at the molecular level, his concept is congruent with what we now know about oncogene activation and gene mutations. In fact, the metabolic patterns described by Weber in such exquisite detail represent the phenotypic exterior that reflects the sequential and cumulative changes in the internal genetic machinery of the cell as proposed by Vogelstein (discussed later).

The Oncodevelopmental Concept

This concept views cancer as an aberration of developmental biology, central to which is the abnormal expression of normal genes. This abnormality is manifested by the presence in neoplastic tissues of proteins that normally are produced during embryonic development but are suppressed in mature differentiated tissues. The seminal discoveries that led to this concept include the presence of α-fetoprotein in hepatic cancer (*19*), of carcinoembryonic antigen (CEA) in colonic cancer (*20*), and of placental alkaline phosphatase found by Fishman and his colleagues (*21, 22*) in several different types of cancer. This reexpression of normal developmental genes by neoplastic cells may potentially be explained by the view that cells with this phenotype, characteristic of an earlier stage of differentiation, randomly persist during neoplasia, a notion consistent with the cell rest theory of carcinogenesis (see below). Alternatively, such reexpression may be a consequence of mutations or deletions in cells that had completed the differentiation program and were fully mature before these genomic alterations. It is unlikely that the ability to synthesize an oncodevelopmental gene product is mechanistically related to neoplasia or confers a selective advantage on cancer cells. Moreover, similar alterations in gene expression occur in cells regenerating after injury, in line with the fact that this

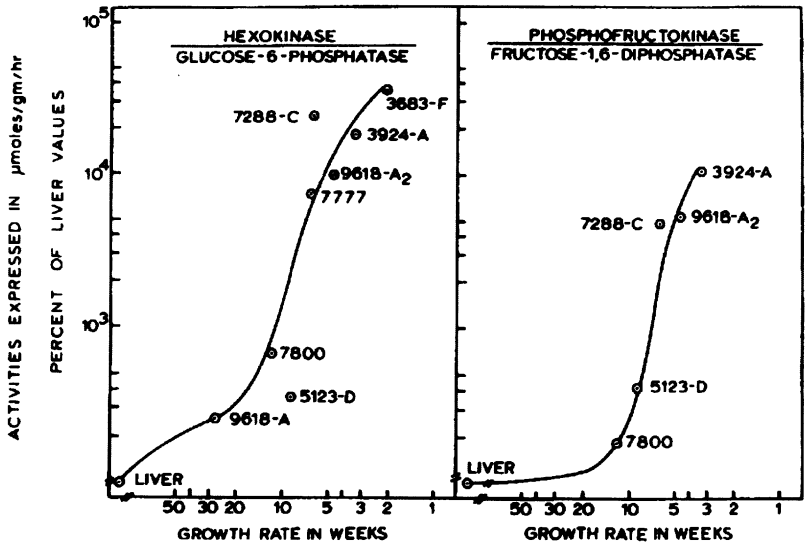

Fig. 1. Correlation of the activity ratios of opposing glycolytic (hexokinase and phosphofructokinase) and gluconeogenic (glucose-6-phosphatase and fructose-1,6-diphosphatase) enzymes with growth rates of various rat hepatomas
From: Weber (*18*). Reprinted with permission from Academic Press

Table 2. Carbohydrate Metabolism: Phenotypic Evidence for Reprogramming of Gene Expression in Neoplasia

Synthetic enzymes	Key gluconeogenic enzymes are decreased
	Glucose-6-phosphatase, fructose, 1,6-diphosphatase, phospho*enol*pyruvate carboxykinase, pyruvate carboxylase
Catabolic enzymes	Key glycolytic enzymes are increased
	Hexokinase, phosphofructokinase, pyruvate kinase
Metabolic imbalance	Ratios of key glycolytic/key gluconeogenic enzymes are increased
Isoenzyme shift	High K_m isoenzymes are decreased
	Glucokinase, liver-type pyruvate kinase
	Low K_m isoenzymes are increased
	Hexokinase, muscle-type pyruvate kinase
Relation to malignancy	Alterations are covariant with growth rate, a malignancy-linked imbalance
Biological role	Imbalance in glycolytic/gluconeogenic enzymes leads to increase in glycolysis, isoenzyme shift leads to decreased responsiveness to physiological controls, both conferring selective advantages to cancer cells

Source: Adapted from Weber (*18*).

regeneration during the recovery of tissues from injury occurs as a consequence of the proliferation of undifferentiated cells and of their entry into the differentiation program that generates mature, phenotypically adult cells. However, it is a statistically established generalization that neoplasms expressing oncodevelopmental proteins, being more primitive, are also more malignant and tend to be more anaplastic than tumors in which the majority of cells do not express these proteins. These ideas have given rise to the application of tumor markers for evaluating the grade of malignancy of tumors and the prognosis of those patients in whom they occur (*23*). The most compelling exponent of the oncodevelopmental concept and its implications, both biological and diagnostic, is William H. Fishman. His contributions have profoundly influenced the management of cancer patients, and his work has provided a rigorous structure for the biological basis of these marker proteins (Table 3).

The Metastatic Phenotype

This is the ultimate stage in the progression of a cancer cell. It requires further mutational events beyond those necessary for the production of tumors that are merely locally invasive. Cells with metastatic potential grow and evolve from among cellularly diverse subpopulations present in such tumors (*24*). To spread to distant sites, such tumor cells have to enter the interstitial stroma and invade the walls of blood vessels, thereby entering the circulation. On reaching the target organ, they adhere to the luminal surface of endothelial cells, stimulating the latter to retract and expose the underlying basement membrane, for which many tumor cells possess cell-surface-matrix receptors. Using a variety of proteases, tumor cells are able to digest the basement membrane, exit the venule or capillary, and invade the surrounding tissue.

Among the phenotypic features facilitating metastasis are the following:

Angiogenesis. This is the ability to stimulate proliferation of small blood vessels through various growth factors (*25*).

Invasion. This occurs by virtue of chemoattractants, autocrine motility factors, receptors facilitating attachment to blood and lymphatic vessels, and an adequate complement of degradative enzymes.

Arrest and extravasation. This involves endothelial exposure, followed by its retraction, dissolution of basement membrane, and entry into the tissues as described

Table 3. Neoplasms of Counterpart Developmental Tissues and Their Marker Proteins

Neoplasms	Marker proteins
A. Gamete neoplasms (seminomas, germinal neoplasms)	α-Fetoprotein
	Placental alkaline phosphatase
B. Embryoblast tumors (embryonal carcinoma)	α-Fetoprotein
	Chorionic gonadotropin
C. Three-germ-layer tumors (teratocarcinoma)	α-Fetoprotein
	Chorionic gonadotropin
	Placental alkaline phosphatase
D. Extraembryonic tumors	
Trophoblastic neoplasms	Chorionic gonadotropin
	Placental lactogen
	Placental alkaline phosphatase
	Pregnancy-specific glycoprotein (SP_1)
	Placental protein 5 (PP_5)
Yolk sac neoplasms	α_1-Antitrypsin
	α-Fetoprotein
E. One-germ-layer tumors	
Ectodermal tumors	
Prostate cancer	Acid phosphatase
Breast carcinoma	α-Lactalbumin
	Casein
	Estrogen receptor
	Placental lactogen
Endodermal tumors	
Colorectal and other gastrointestinal cancers	CEA
Mesodermal tumors	
Skeletal muscle tumors	Myoglobin
Smooth and skeletal muscle tumors	Myosin
Osteogenic sarcoma	Alkaline phosphatase
Endothelial cell tumors	Factor VIII-related antigen

Source: Adapted from Fishman (*23*). The scheme developed by Fishman includes two further categories: F, endocrine tumors; G, specialized migratory embryonic cell neoplasms, divided into further subclasses, including neural crest cell tumors, gastrointestinal tract endocrine cell tumors, germ neoplasms, and hematopoietic disorders.

above, the relevant factors for each of these processes being listed in Table 4.

Formation of secondaries. Tissue receptors, growth factors, and an ability to invade host defenses, as outlined in Table 4, contribute to this process.

Diagnostic Implications

The application of biochemical tests to cancer diagnosis has consumed the energies of a multitude of investigators, generated a voluminous literature, and sown confusion and uncertainty on a scale that has made the present Conference obligatory. The possibility that a single diagnostic test for all forms of cancer exists and simply awaits discovery was a cherished notion a few decades ago. The more sober hope that a series of individual tests for specific cancers might emerge seemed to have a good prospect, and various groups of scientists appeared to be on the verge of success in many instances. Regrettably, these hopes have fallen upon disappointment and frustration. Few, if any, of the many so-called diagnostic tests for cancer have survived in this role, and those that remain in the laboratory repertoire at this time are valued, if at all, for their potential usefulness in therapeutic monitoring and prognosis. Rather than enter into a broad discussion of these tests, we will focus on a historical perspective by illustrating some of the biochemical procedures that have been explored for their utility in two specific forms of cancer, and will relate these to the conceptual background already presented.

Cancer of the Cervix Uteri

Changes in biochemical constituents of blood and urine are rare in cervical cancer, but its anatomic location has led to the exploration of vaginal fluid as a potential source of biochemical signals for this condition, with enzymes being the constituents most actively investigated (*26–32*). Goldberg and associates conducted an intensive study of this possibility for more than a decade (*33–38*). Beginning with the characterization of enzymes in the tumor tissue in comparison with normal cervical mucosa, they showed that high

Table 4. Tumor–Host Interactions during the Metastatic Cascade

Metastatic event	Possible mechanisms
Angiogenesis	Multiple angiogenesis factors including growth factors
Invasion of local tissues, blood, and lymphatic vessels	Serum chemoattractants, autocrine motility factors, attachment receptors, degradative enzymes
Circulating tumor cell arrest and extravasation	Tumor cell aggregation
Adherence to endothelium	Tumor cell interaction with fibrin, platelets, and clotting factors; adhesion to RGD-type receptors
Retraction of endothelium	Platelet factors, tumor cell factors
Adhesion to basement membrane	Laminin receptor, thrombospondin receptor
Dissolution of basement membrane	Degradative proteases, type IV collagenase, heparanase, cathepsins
Locomotion	Autocrine motility factors, chemotaxis factors
Colony formation at secondary site	Receptors for local tissue growth factors, angiogenesis factors
Evasion of host defenses and resistance to therapy	Resistance to killing by host macrophages, natural killer cells, and activated T-cells; amplification of drug-resistance genes

Source: adapted from Liotta and Stetler-Stevenson (*24*).

concentrations of lysosomal enzymes such as ribonuclease, deoxyribonuclease, and β-glucuronidase were characteristic of these tumors (*33, 34*). Higher concentrations of glycolytic enzymes, e.g., lactate dehydrogenase, and of direct oxidative enzymes, e.g., phosphogluconate dehydrogenase, were also present (*35–38*). Finally, an increase in membrane-related enzymes was also a prominent feature, as illustrated by GGT in Figure 2.

These differences were evident when the vaginal fluid content of cancer patients was compared with that of normal healthy women (Figure 3). Despite the fact that these procedures were very accurate in diagnosing invasive cancer, lent themselves to mechanization and automation, and were relatively cheap to perform, they never succeeded in reducing dependency on cytological examination as the standard screening procedure for diagnosis of cervical cancer. This could be attributed to their poor sensitivity in detecting carcinoma in situ, a preinvasive lesion believed in many cases to remain localized and to have a more benign outcome and a different natural history than invasive cervical cancer.

Cancer of the Colon

Many investigations have been conducted on tissue removed at operation, and the results have given considerable insight into the alterations in gene expression that, if not quite causal, are important in the biology of colonic cancer and frequently correlate with clinical aspects of the disease (Table 5). For example, tumors expressing steroid receptors tend, on the whole, to be more responsive to therapy than those without, reminiscent of the well-known behavior of breast cancers. Enzymes of polyamine biosynthesis are greatly enriched in colonic cancers, in dysplastic polyps, and even in the adjacent apparently normal mucosa of individuals with familial polyposis coli.

Altered expression of blood-group antigens is a prominent feature of colon cancers. These changes are characterized by the disappearance of antigens normally expressed in mature adult tissue and the reappearance of products expressed in the fetus, suggesting a reactivation or derepression of fetal gene function. In addition, some blood-group glycolipids synthesized by tumors are blocked at some point in the synthetic pathway; these glycolipids therefore behave as incompatible blood-group antigens foreign to the host (*77*).

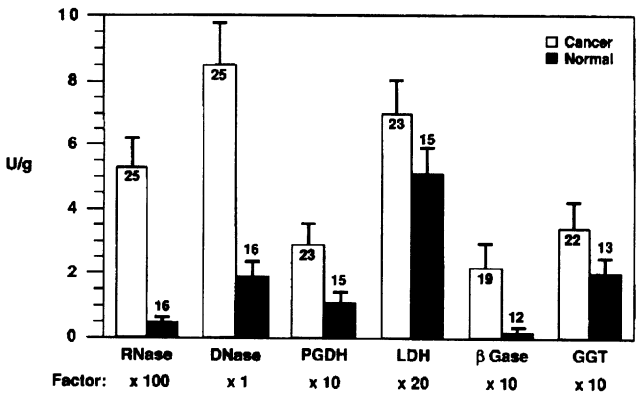

Fig. 2. Activities of various enzymes as U/g of cervical tissue in biopsy samples of patients with invasive carcinoma of the cervix uteri and in histologically normal samples from subjects with benign cervical lesions

Number of samples for each enzyme as indicated at *bars*. Multiply *y*-axis units by the factor indicated for each enzyme to obtain the true values as mean ±SE. Compiled from published and unpublished data of Goldberg et al. (*33–38*). PGDH, 6-phosphogluconate dehydrogenase; LDH, lactate dehydrogenase; β-Gase, β-glucuronidase

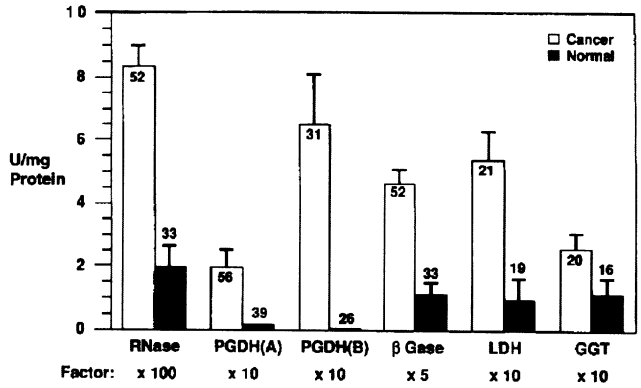

Fig. 3. Activities of various enzymes, U/mg of protein, in vaginal fluid samples collected from patients with invasive carcinoma of the cervix uteri and from subjects with benign cervical lesions

Number of samples for each enzyme as indicated at *bars*, enzyme abbreviations as in Fig. 2. PGDH(A) and (B) refer to intracellular supernate and vaginal fluid plasma (extracellular), respectively. RNase, β-Gase, and GGT were assayed in homogenized cells sedimented from the vaginal fluid by centrifugation, and LDH was assayed in the intracellular supernate of these cells

The synthesis of oncogene products as a result of activation by different mechanisms such as gene amplification and increased gene transcription and gene translocation, and the increased stability of the respective oncogene products as a result of mutation or decreased catabolism are being actively explored for all tumors, and colon cancer is no exception. A more detailed account of the significance of these changes will be discussed in the next section.

Serum tests in patients with colonic cancer have been dominated by assays of CEA. Table 5 summarizes some of the earlier literature on the use of this marker. The assertions of utility in Table 5 are far from unanimous. The value of CEA in staging is limited (*67*). Its prognostic accuracy appears to be relatively high only in Dukes' stage C disease (*78, 79*), and in a large series of 333 patients Lewi et al. (*80*) found no predictive relationship between preoperative CEA concentrations and survival. Its role in "second-look surgery" is very controversial, given the false-positive rate of ~15%, and, although the true-positive rate approximates 80%, few patients prove to have operable recurrences (*65, 81–83*). CEA values are not helpful in assessing the response to chemotherapy (*84*) or to radiation (*85*).

As with most other solid tumors, numerous enzymes have been used to evaluate patients with colonic cancer, mainly for the purpose of detecting spread to the liver (see references *86–89* for review). Alkaline phosphatase and GGT have been standard aids in this assessment; measurement of their total serum activity usually suffices, but some diagnostic improvement may follow the

Table 5. Biochemical Markers of Neoplasia in Tissue and Serum of Human Subjects with Colonic Cancer

Test	Utility	References
Tissue		
Steroid receptors		
Estrogen receptor	Suggestive of better prognosis	*39–41*
Androgen receptor	None	*42*
Polyamine biosynthesis		
Ornithine decarboxylase	Identifies benign lesions at greater risk of malignancy	*43, 44*
S-Adenosyl methionine decarboxylase	As above	*44*
Blood group antigens		
A & B isoantigens	Loss from proximal colon in which normally expressed, reexpression in distal colon in which normally absent	*45–47*
Lewis^a antigen	Decreased in poorly differentiated and metastatic cancers	*46–48*
Lewis^b antigen	Expressed in almost all colon cancers but only 20–45% of normal tissues	*46–48*
Lewis^x antigen	Present in differentiated but not poorly differentiated cancers or normal colonic tissues	*49*
Lewis^y antigen	Absent from adult colon; present in cancer and polyps, correlating with dysplasia	*50*
H antigen	Marker of dysplastic premalignant lesions	*51*
Growth factors		
TGF α and β	May stimulate stromal cell growth	*52, 53*
Oncogenes		
ras	Mutations present in 30–40% of colon cancers and 60% of colonic adenomas >1 cm	*54–58*
	Inversely related to dedifferentiation	*59–61*
c-*myc*	Does not correlate with disease-free interval or overall survival	*62*
Serum		
Carcinoembryonic antigen	Useful in staging	*63*
	Correlates with prognosis	*64–67*
	Index of tumor burden	*64*
	Predictive of recurrence	*63, 68*
Enzymes		
GGT	Detects hepatic metastases	*69–71*
Lactate dehydrogenase	Elevated in >50% of cases without metastases	*70*
Alkaline phosphatase	Detects hepatic metastases	*71, 72*
	Predicts survival	*73*
Acute-phase proteins		
α-Antiprotease	Predicts metastases	*74, 75*
α_1-Acid glycoprotein	Predicts metastases	*74*
	Index of tissue fixation	*76*
Haptoglobin	Predicts recurrence	*74*
Prealbumin	Nutritional index	*74*
Serum hexose	Useful in diagnosis, predicts recurrence	*75*
C-reactive protein	Index of tissue fixation	*76*

specific determination of high-molecular-mass forms of these enzymes (*71*).

It may appear strange that acute-phase proteins, which are produced by the host and not by the tumor, should have exercised a fairly prominent role in the management and evaluation of cancer patients. However, as is apparent from Table 5, some of these tests give useful information about tumor–host interactions. They also lend themselves to data massage in the form of discriminant function analysis, and some useful diagnostic functions have been generated by this kind of exercise (*90*).

Theories and Mechanisms of Neoplasia

The most influential view of cancer as a biological process in the immediate post-World War II era was the proposal, advocated by Jesse P. Greenstein, that the cancer phenotype represented the progression of mature differentiated cells towards a final common pathway characterized by unrestrained growth, proliferation, metastasis, and liberation from the constraints that normally prevent these processes from occurring. Viewed as an intrinsic behavior to which all cells are naturally prone as they age, the process could be accelerated by many predisposing factors of a genetic or environmental nature, including exposure to carcinogens, radiation, viruses, and chronic tissue injury (*91*). Much as this concept captured the imagination of cancer investigators, it did not easily lend itself to direct experimental proof. Consequently, over the succeeding two decades, various alternative hypotheses or embellishments of

earlier theories were generated. In 1974, Busch (*92*) summarized those that had dominated research in cancer up to that time (Table 6). We point out that these approaches are by no means mutually exclusive and, even if they are not generalizable, they may still be valid for specific types of cancer. Nor can it be asserted that current knowledge about the genetics of cancer has rendered these concepts obsolete. In fact, the reverse is the case. The more we discover about carcinogenesis, the more perceptive these views become.

To deal with these theories in sequence, although cellular anoxia is not a cause of cancer, it is a very common consequence of neoplastic change. Chromosomal changes and point mutations are crucial events in carcinogenesis and lie at the heart of all current genomic models. A failure to eliminate tumor cells, whether as a consequence of impaired immunity or of a failure in genomic policing (*102*), is a seminal element of current cancer theory. Although Potter originally proposed the "deletion hypothesis" with negative regulators of DNA-synthesizing enzymes in mind (*96*), the concept fits very well with the known effects of *p53* suppressor gene mutation or deletion and the role of this gene in carcinogenesis. "Activation of latent cancer DNA" is simply a more primitive form of the phrase "oncogene expression." The notion that precancerous cells are present in many tissues is central to the most completely established model of carcinogenesis (*103*), which we will further elaborate later. Insertion of viral genomic material is a key mechanism in virus-induced carcinogenesis, and what we currently know about oncogenes serves to elaborate rather than to disprove the original concept. Pitot's "membron" concept (*101*) is still valid in the sense that membrane abnormalities are a virtually constant feature of cancer cells, although not the fundamental cause of the condition. Finally, genomic derepression and reprogramming are well-established phenomena that, as mentioned earlier, account for many of the phenotypic changes in cancer cells, even though they are the consequences of events rooted at a more profound level of the reproductive machinery of the cell rather than the primary cause of cancer per se.

It is indeed quite remarkable that the more sophisticated our molecular techniques have become, the more accurate and valid these older observations and views appear to be. Research in the past two decades has succeeded in providing a description and an explanation for these postulated events at a level that merely serves to reinterpret these concepts in contemporary terminology. In the following sections, we look at two models of carcinogenesis that are worthy of more detailed consideration.

Chemical Carcinogenesis

It is to James and Elizabeth Miller that we owe much of our fundamental knowledge about this process (*104*), although several investigators such as Nery (*105*), Pitot (*106, 107*), and Farber (*108, 109*) also advanced our knowledge of the relevant processes. Most of the experimental observations stem from investigations performed in laboratory animals, the commonest models being hepatic and skin carcinogenesis in the rat and mouse, respectively. Information from such models may not be relevant to the commonest spontaneously occurring tumors in humans, but they are obviously relevant to those that have been established to arise as a consequence of environmental insults such as cigarette smoking (lung, and possibly bladder and breast cancer), ultraviolet radiation (skin cancer), and various industrial cancers endemic to workers in the manufacture of dyes and other chemicals (bladder and scrotum cancer). Some of these factors are listed in Table 7, and the possible mechanisms are presented in Table 8.

Experiments in this field have led to the recognition of three distinct stages. The first, *initiation*, is irreversible, and the initiated cells retain a "memory" characteristic of the process. The resulting cell has the potential to develop into a clone of neoplastic cells but will not necessarily do so. The second stage, *promotion*, is distinguished from initiation by its reversibility. The existence of promoted cell populations depends on the

Table 6. Major Theories of Neoplasia

Concept	Author (ref.)	Description
Abnormal cell respiration	Warburg, 1926 (*93*)	Cancer may arise as a result of cellular anoxia, which causes excessive glycolysis.
Somatic mutation theory	Boveri, 1929 (*94*)	Chromosomal changes or point mutations cause alteration in cellular function.
Defects in immune surveillance	Green, 1954 (*95*)	Carcinogenic substances alter the immune cell response to tumors, with failure to eliminate tumor cells.
Deletion hypothesis	Potter, 1957 (*96*)	Cytoplasmic reactions may control cell division; carcinogens may cause loss of these control reactions.
Activation of latent cancer DNA	Busch, 1962 (*97*)	Cancer DNA present in all cells but normally repressed; various stimuli result in expression of this DNA.
Cell rest theory	Cohnheim 1889 (*98*); Osgood, 1964 (*99*)	Precancerous or "resting" cells present in many tissues are activated to cancer cells by oncogenic stimuli.
Viral genome insertion	Temin, 1965 (*100*)	Whole viral DNA or DNA produced by RNA-dependent DNA polymerase inserted into cell genome.
Membron hypothesis	Pitot, 1969 (*101*)	Messenger RNA is fixed to specific membrane sites that are abnormal in cancer cells.

Source: Adapted from Busch (*92*).

Table 7. Factors and Agents Convincingly Demonstrated to Be Associated with Predisposition to Specific Forms of Cancer

Agent	Specific neoplasm
Life-style factors	
Alcoholic beverages	Esophagus, liver, pharynx
Betel chewing	Mouth
Dietary factors (fat, protein, calories)	Breast, colon, endometrium, gallbladder
Sexual promiscuity	Cervix uteri
Tobacco smoking	Mouth, pharynx, larynx, lung
Occupational exposure	
Arsenic	Lung
Asbestos	Lung
Aromatic amines	Bladder
Cadmium	Lung
Nickel	Lung
Rubber industry	Leukemia, bladder
Soots, tars, and oils	Skin, lung, bladder, gastrointestinal tract
Medications	
Inorganic arsenicals	Skin, liver
Azathioprine	Lymphoma, reticulum cell sarcoma
Chloramphenicol	Leukemia
Diethylstilbesterol	Vagina
Phenacetin	Renal pelvis

Source: Adapted from Pitot (*107*).

Table 8. Possible Mechanisms of Chemical Carcinogenesis

Genetic mechanisms, which result in modifications of the DNA genome
Direct modification of the DNA
Modification of RNA subsequently transcribed into DNA and integrated into host DNA
Alterations of molecules other than DNA that decrease the fidelity of DNA copying
Epigenetic mechanisms
Changes in DNA transcription (including integrated virus genomes and oncogenes)
Proliferation and progression toward malignancy of previously existing preneoplastic or neoplastic cells
Increased superoxide formation
Activation of protein kinase C

Source: Expanded from Miller and Miller (*104*).

continued administration of the promoting agent, but the maximal effect depends on the number of cells already initiated, and therefore on the dose of the initiating agent. The final stage, *progression*, is irreversible and is accompanied by clearly recognizable genomic alterations; at this stage, the formation of discrete tumors (benign or malignant) becomes evident. Before this point, initiated cells can be recognized only by subtle phenotypic changes, such as a dramatic increase in GGT content, as in the case of hepatic and thyroid carcinogenesis (*110–112*). Some agents, termed complete carcinogens, are able to bring about all three stages; others, termed incomplete carcinogens, act merely as initiating agents. Some compounds that are complete carcinogens at high doses are incomplete carcinogens at low doses. Still others, such as urethane, are complete carcinogens for certain tissues, but incomplete for others.

This model of carcinogenesis has been most effective in explaining the development of tumors due to environmental contaminants and radiation. Conceptually, however, it is linked very clearly to the next model.

The Vogelstein Model

This is the most recent model to be proposed concerning the origin and development of cancer cells (*103*). Unlike most of the earlier models, it is based on observations in human cancer, the best-documented being cancer of the colon. In fact, the elements in this hypothesis are really not new; what is novel is the specific description of the events leading to cancer in human tissues and the characterization of the various abnormalities that contribute to this process (Figure 4). As in the instance of some of the earlier models, the hypothesis provides a rational basis for the application of certain laboratory procedures in the diagnosis of cancer in general and the evaluation of prognosis in particular cases.

The background to this hypothesis has been provided by the recognition of a range of genomic alterations encountered in a high proportion (if not all) of human cancer cells. The first of these, *ras* gene mutation, occurs in 50% of colorectal carcinomas and in adenomas in the same tissue >1 cm diameter, suggesting that such mutations may be the initiating event in a subset of such tumors (Figure 5). This observation also suggests that adenomas with such mutations are more likely to become malignant than those without.

The second series of events typically present in colonic neoplasms is loss of tumor-suppressor genes (*113, 114*). Those regions of the genome thus affected include chromosome 5q (20–50% of colorectal carcinomas in patients without polyposis), chromosome 17p (75% of colorectal carcinomas, although very rarely in adenomas), and chromosome 18q (>70% of carcinomas and 50% of late adenomas). The significance of the 5q region is that this is the locus linked to familial polyposis. Patients with this condition only rarely have deletions of chromosome 5q; presumably mutations rather than allelic losses account for development of polyposis. The 17p region has been identified as the locus containing the *p53* gene. In addition to allelic deletion, point mutations causing amino acid substitutions in the p53 gene product occur frequently in colorectal tumors. Unlike the wild-type gene product, which appears to undergo very rapid catabolism and is undetectable in normal cells, the mutant proteins accumulate and can be detected in the affected cells by immunocytochemical or immunoassay techniques. Although allelic loss and point mutations are independent events, they have been found to coexist not infrequently in colorectal carcinomas.

The 18q region encodes a candidate tumor-suppressor gene termed *DCC*, the protein product of which is homologous with a family of cell adhesion molecules. Al-

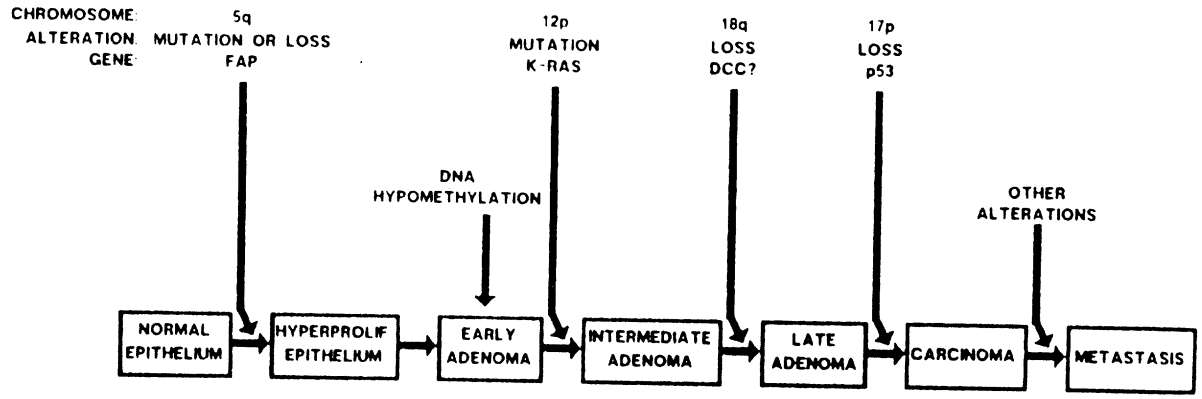

Fig. 4. Genetic model for colorectal tumorigenesis

Tumorigenesis proceeds through a series of genetic alterations involving oncogenes (*ras*) and tumor-suppressor genes (particularly those on chromosomes 5q, 17p, and 18q). Patients with familial adenomatous polyposis (FAP) inherit a mutation on chromosome 5q, an alteration that may be responsible for the hyperproliferative epithelium in these patients. In tumors arising in patients without polyposis, the same region may also be lost and (or) mutated at a relatively early stage of tumorigenesis. Hypomethylation is present in very small adenomas in patients with or without polyposis, an alteration that may lead to aneuploidy, resulting in the loss of suppressor gene alleles. *ras* gene mutation appears to occur in one cell of a preexisting small adenoma and, through clonal expansion, produces a larger and more dysplastic tumor. The chromosomes most frequently deleted include 5q, 17p, and 18q; the putative target of the loss event (i.e., the tumor-suppressor gene) on each chromosome is indicated as well as the relative timing of the chromosome loss event. The order of these changes is not invariant, and accumulation of these changes, rather than their order with respect to one another, seems most important. From: Fearon and Vogelstein (*103*). Reprinted with permission from the authors and Cell Press

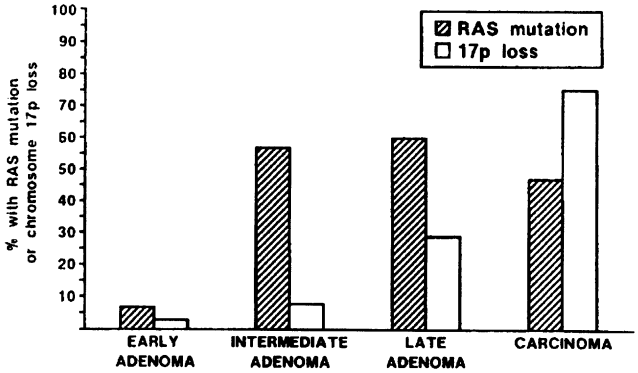

Fig. 5. Frequency of *ras* gene mutations and chromosome 17p deletions in colorectal tumors

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tered expression of this gene may therefore be expected to interfere with cell–cell or cell–matrix interactions.

Gene amplification of chromosome 12 results in overexpression of a protein, MDM2, which is able to complex with p53 and inactivate its tumor-suppressing activity. Similar inactivation occurs when p53 binds with a number of viral products and oncoproteins such as simian virus 40 T (SV40 T), adenovirus E1b, and papilloma virus E6 antigens.

A range of somatic alterations have been found with high frequency in colorectal tumors. Reduced DNA methylation causes genomic instability, reduced chromosome condensation leading to mitotic nondisjunction, and allelic losses. Overexpression of c-*myc* and increased tyrosine kinase activities are also encountered frequently in colorectal carcinomas.

The Vogelstein hypothesis incorporates the following proposals:

1. Most, if not all, colorectal carcinomas arise from preexisting benign adenomas.

2. Colorectal tumors arise from activation of oncogenes coupled with inactivation of tumor-suppressor genes, both of which occur as a result of mutational events.

3. Most carcinomas require at least five or more genetic alterations, whereas adenomas arise subsequent to fewer mutations. This multistep process is analogous to the three steps of initiation, promotion, and progression described earlier. An example of the necessity for multiple genetic alterations in the genesis of adenomas and their progression to cancer is provided in Figure 6, in which *ras* gene mutations and allelic deletions of chromosomes 5q, 17p, and 18q were studied in 53 carcinomas, 27 early adenomas, 12 intermediate adenomas, and 14 late-stage adenomas (*103*).

4. The total accumulation of changes rather than their precise sequence with respect to one another determines whether tumorigenesis occurs and whether it

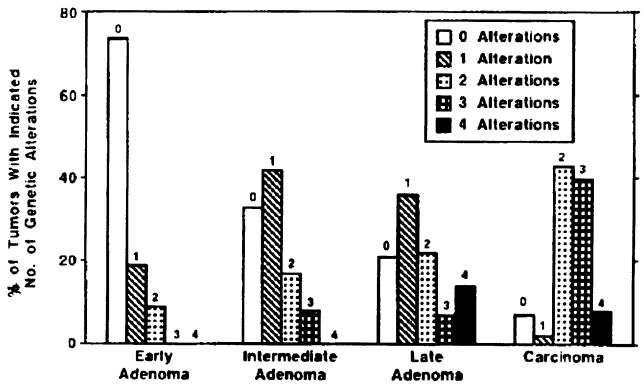

Fig. 6. Number of genetic alterations in colorectal tumors

Four genetic alterations (*ras* gene mutations and allelic deletions of chromosomes 5q, 17p, and 18q) were studied in each colorectal tumor; thus, each tumor could have had none, one, two, three, or all four of these alterations. The tumors included 53 carcinomas, 14 late-stage adenomas, 12 intermediate adenomas, and 27 early adenomas. From: Fearon and Vogelstein (*103*). Reprinted with permission from the authors and Cell Press

will be benign or malignant. However, certain changes do have a tendency to appear in a sequential order. Thus, as shown in Figure 5, *ras* mutation tended to occur earlier than loss of p53 suppressor activity, as determined by chromosome 17p deletions.

5. The hypothesis emphasizes the primacy of tumor-suppressor gene inactivation, which, even in the heterozygous state, is able to exert a "dominant negative" effect. This aspect of the hypothesis has been strongly emphasized by Lane (*102*), who refers to p53 protein as the "guardian of the genome."

To better understand these concepts, a word of explanation is necessary. p53 is believed to function as a tetramer that binds to p53-specific genomic sites to stimulate the expression of genes that inhibit growth and (or) invasion (Figure 7). Loss of one or both *p53* alleles may occur in some tumors by chromosomal defects or deletions (Figure 7-1). Truncation of p53 by a "nonsense" mutation may cause loss of the oligomerization domain and prevent the formation of tetramers (Figure 7-2). This will reduce the number of functional tetramers by about half. Paradoxically, a "missense" mutation allows the abnormal peptide to participate in the formation of tetramers; however, these tetramers will be nonfunctional and will result in a much greater impairment of growth regulation, because mutant wild-type tetramers do not function normally. This dominant negative effect may be enhanced by the greater stability of the mutant protein, which will therefore attain a higher intracellular concentration than the wild-type protein will (Figure 7-3). As already mentioned, a missense mutation of one *p53* allele often coexists with

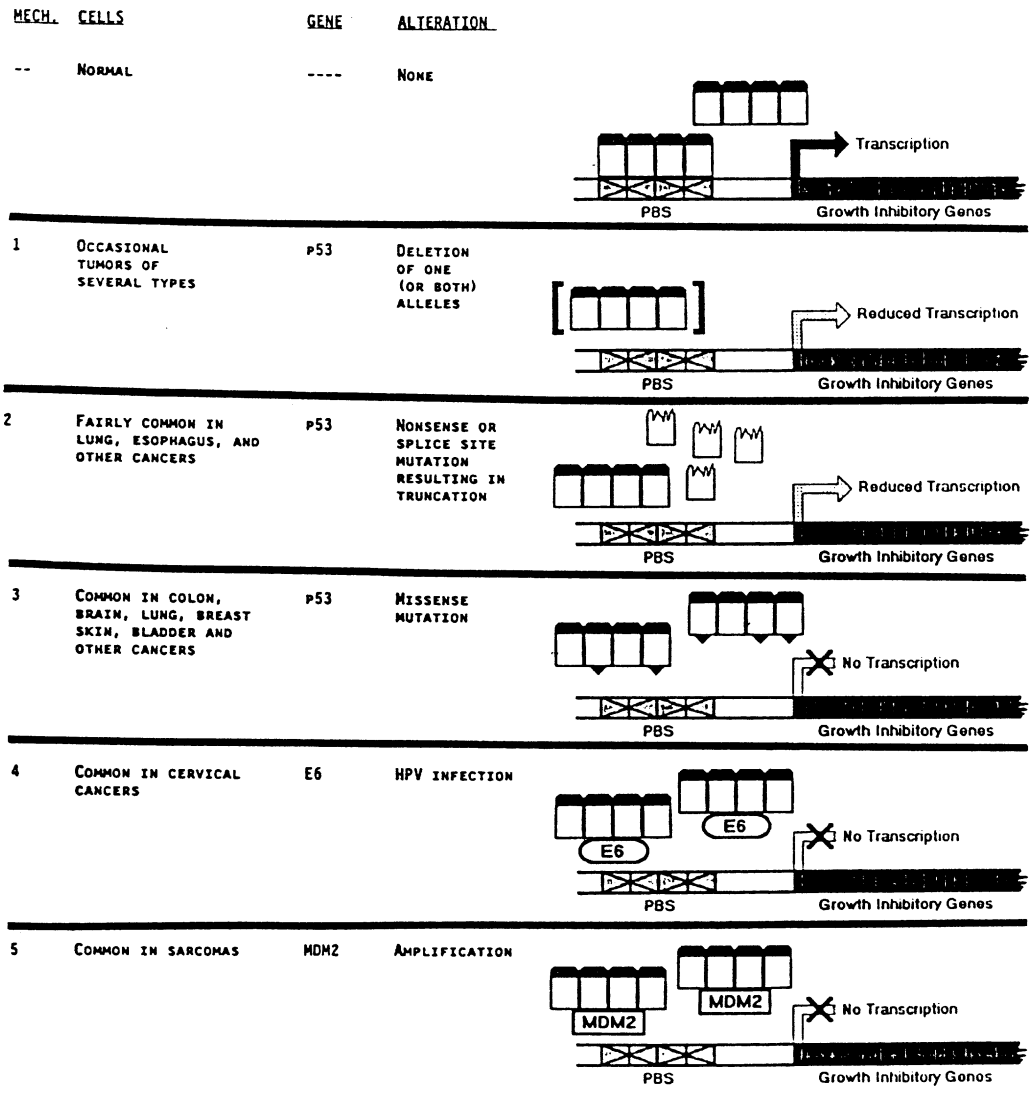

Fig. 7. p53 inactivation mechanisms

p53 is postulated to bind as a tetramer to a p53-binding site (PBS) and activate the expression of adjacent genes that inhibit growth and (or) invasion. Deletion of one or both *p53* alleles reduces the expression of tetramers, resulting in decreased expression of these genes (mechanism 1). Mutations that truncate the protein do not allow oligomerization, thus resulting in a similar reduction of p53 tetramers (mechanism 2). Missense mutations resulting in dominant negative effects result in an even greater reduction of functionally active tetramers (mechanism 3). By binding to p53, the expression of E6 (mechanism 4) and increased expression of MDM2 (mechanism 5) result in functional inactivation of p53. It is not known whether E6–p53 and MDM2–p53 complexes inhibit binding to p53-binding sites, or whether they allow binding to p53-binding sites but inhibit transcriptional activation. E6 may also degrade p53 through ubiquitin-mediated proteolysis. HPV, human papillomavirus. From: Vogelstein and Kinzler (*114*). Reprinted with permission from the authors and Cell Press

deletion of the other; consequently, there is a complete absence of any wild-type p53 tetramers in such tumors. Finally, the overexpression of gene products such as E6 and MDM2, which form strong complexes with p53, prevents its growth-inhibitory function, either by blocking its binding to the specific regulatory sites on the genome, or by inhibiting transcriptional activation after binding occurs (Figure 7-4 and -5).

It is now known that p53 mediates the arrest of the cell cycle at G_1 phase. According to Lane's proposal (*102*), if DNA is damaged, the p53 accumulates, blocks replication, and allows time for repair to take place; if this repair fails to happen, p53 may initiate cell death. This process does not occur in tumor cells, in which p53 is absent or inactivated (by mutation of the gene or by binding to host or viral proteins). These cells, which are genetically less stable, will develop mutations and chromosomal rearrangements, proliferating by clonal expansion. DNA viruses must inactivate p53 (the policeman) if they wish to replicate their DNA after damaging the cell, because this replication occurs in S phase, which will not be reached if p53 function is intact.

Implications of the Vogelstein/Lane Model

Given that the predisposition to form colorectal tumors can be inherited, identification of individuals with defective tumor-suppressor genes on (e.g.) chromosomes 5q, 17p, and 18q may enable preventative surgery to be carried out, or at least lead to an informed selection of those subjects in whom surgery is more imperative than others. Before rushing to embrace this notion, one should recall that we already have, in ornithine decarboxylase, a marker for enhanced likelihood of malignancy in patients with polyposis coli (*43, 44*). There is as yet no evidence that these chromosomal alterations are as frequent as increases in ornithine decarboxylase activity or as accurate a predictor of malignancy; nor have the relative costs of both procedures been ascertained.

As mentioned above, mutant p53 protein accumulates in tumors, whereas the normal wild-type protein does not. Several studies have evaluated p53 as a prognostic index in cancer patients. Thor et al. (*115*) have recently shown, by analysis of nearly 500 tissue samples taken from patients with invasive ductal carcinoma of the breast, that at every stage (lymph node-negative, lymph node-positive, etc.), positivity for p53 mutation reduced both metastasis-free and overall survival (Figure 8). Similar results have been described in prostatic carcinoma (*116*). Enthusiasm for these findings has to be tempered by the realization that for many tumors (e.g., those of lung and colon), high concentrations of serum CEA are associated with similar or even greater reductions in survival rates, as is also the case for a number of other easily measurable tumor markers. Aside from the issue of cost, standardization of these earlier serum assays is well advanced, and interlaboratory reproducibility has been steadily improving. As yet, the immunocytochemical techniques used to test for p53 mutations are technically unsatisfactory, poorly standardized, and not reproducible between laboratories (*117*). New quantitative immunological techniques for measuring p53 protein in tumor extracts are now evolving (*118*). Using such procedures, investigators have found a strong negative association between the presence of mutant p53 and the absence of steroid hormone receptors in breast tumor tissue. Although these and other reports suggest that p53 may be an independent breast cancer prognostic indicator, with the likelihood of being introduced into clinical practice in the next 1–2 years (*119*), investigators will have to clearly demonstrate that the test provides additional information rather than merely being an expensive alternative to steroid receptor analysis.

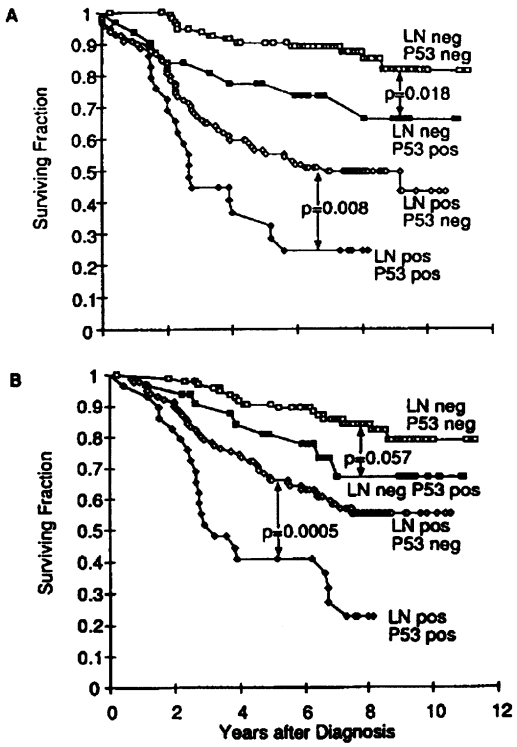

Fig. 8. Kaplan–Meier survival curves by lymph node (LN) and p53 protein status

The number of patients represented by each curve is as follows: (*A*) metastasis-free survival: lymph node (LN)−, p53− (n = 96); LN−, p53+ (n = 31); LN+, p53− (n = 98); LN+, p53+ (n = 28). (*B*) Overall survival: LN−, p53− (n = 96); LN−, p53+ (n = 32); LN+, p53− (n = 102); LN+, p53+ (n = 29). *P* values are for pairwise comparison of survival curves based on the Cox–Mantel test. Symbols on dropping portions of lines represent failures (i.e., metastasis or death); each symbol represents one patient. From: Thor AD, et al. (*115*). Reprinted with permission

Optimistic pronouncements have been made to the effect that new therapeutic agents might be developed to selectively inactivate mutated gene products, (e.g., *ras*) or to restore the normal action of defective suppressor genes (see reference *120* for a review). However, implementation of such therapies is nowhere near the progress already made with readily available anticancer drugs. Considerable excitement has accompanied attempts to exploit for diagnostic purposes the information implicit in the Vogelstein model, employing sophisticated molecular genetic techniques. It is well to put these into a meaningful perspective and to carefully weigh their practical utility.

Ras *oncogene mutations*. Recently, an important report was published describing identification of the *ras*

mutation in DNA extracted from the stool in eight of nine cases with colonic neoplasms (*121*). In evaluating the practical significance of this announcement, one should be aware that two of the positive results were found in patients with adenomas, whereas the false-negative subject was the only early carcinoma (Duke stage A) among the nine. The six positive results in carcinoma subjects were from three Duke B and three Duke C patients. More worryisome is the fact that the 9 tumors with *ras* gene mutation were drawn from among 24 tumor patients examined, in 15 of whom this mutation was absent in the tumor and therefore not sought in the stool. Although these observations have the potential to lead to the development of diagnostic tests, reality is a long way off.

p53 *gene mutation.* A year earlier, Sidransky et al. (*122*) published the results of an investigation in 18 patients with bladder cancer, 11 of whom had mutations of the p53 gene. Using the polymerase chain reaction and oligomer-specific hybridization, they identified equivalent mutations in a few percent of the cells present in urinary sediment of three patients tested. Why were only 3 of the 11 subjects tested? The report offers no explanation for this reticence. Is the test more sensitive and more efficient than cytological examination? We do not know the answer. Future research must focus on issues of this nature if the results are to lead to the introduction of practically useful and enduring laboratory procedures.

Antibodies to p53 gene product. The above two approaches depend on analysis of cellular material that is not always accessible, particularly in solid tumors that do not drain to the exterior the way those of tissues forming the gastrointestinal and urinary tracts do. A conceptual advance has therefore been the realization that antibodies to p53 protein may be found in the circulation in some cancer patients. This was first described for breast cancer, being present in 15% of such patients, predominantly in those with high histological grade, absence of hormone receptors, and therefore a particularly unfavorable prognosis (*123*). A subsequent study involving a more widely applicable technique reported a somewhat lower incidence (*124*). Newer studies on p53 antibody concentrations in various groups of cancer patients reveal that certain cancers (e.g., ovarian, colon, breast) have much higher incidences of these antibodies than do others (*125*). It remains to be seen whether this serological test for cancer has any potential for screening or diagnosis of some types of tumors.

Conclusion

Cancer research has never been so exciting as at the present time. New concepts and the explosive growth in knowledge of the human genome and how it functions have spawned an array of molecular procedures whose potential is awesome but which at present are of limited practical utility. Table 9, which lists some of the commoner oncogenes and the tumors with which they are most frequently associated, indicates the scope of what lies in store for future exploitation and evaluation in this field. Science has never been immune to the spell of novelty. However, only when the new becomes mundane will its role in the management of cancer patients have earned an honorable place in the ongoing history of oncology. To conclude on an optimistic note, we emphasize that the current understanding of cancer pathogenesis at the molecular level will, in the foreseeable future, give rise to new ways for cancer prevention, diagnosis, treatment, and monitoring. Currently, however, the major advances in molecular biology have not as yet significantly helped the cancer patient. Small battles have been won, but the war is not over.

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Table 9. Representative Examples of Oncogenes with Potential Clinical Application

Tumor	Locus	Applicable feature	Application
Acute lymphocytic leukemia	*bcr-abl*	Distinctive translocation	Distinguish from chronic myelogenous leukemia
Adenocarcinoma of lung	K-*ras*	Point mutations	Prognosis
Chronic myelogenous leukemia	*bcr-abl*	Translocation breakpoint	Diagnosis in absence of Philadelphia chromosome
Carcinoma of bladder	p53	Point mutations	Diagnosis
Carcinoma of breast	*erb*B-1	Overexpression	Prognosis
	neu	Amplification	Prognosis
	11q13	Amplification	Prognosis
	11p	Deletion	Prognosis
	myc	Amplification	Prognosis
	p53	Point mutations	Prognosis
Carcinoma of colon	K-*ras*	Point mutations	Diagnosis
	APC	Point mutations	Diagnosis
Myelodysplasia	N-*ras*/K-*ras*	Point mutations	Prognosis and selection of therapy
Neuroblastoma	N-*myc*	Amplification	Prognosis and selection of therapy
Retinoblastoma	*Rb*1	Loss or damage	Detection of predisposition

APC, adenomatous polyposis coli gene.

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