Toward Mechanism-Based Cancer Care

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Cancer, like many other chronic afflictions, is an ancient disease which, despite all the progress in treatment and prevention, remains a powerful threat. In this age of enlightened medical science, what is the fate of a disease so common that nearly 40% of us will experience it? The annual incidence of cancer in the United States approximates 900,000 cases, and there are 400,000 deaths each year. Furthermore, once cancers become metastatic, it is difficult to cure even the most common tumors, such as those of epithelial origin. Treating cancer remains one of medicine’s most difficult challenges.

High incidence notwithstanding, death rates from some tumors have started to decline during the past decade, as reflected in data from the National Cancer Institute. During this period, death rates from lung, colon, breast, prostate, and stomach cancer have all decreased by at least 1%. Lung cancer mortality has decreased in US men as the result of prevention, primarily efforts to reduce smoking. Early detection methods have contributed to reductions in death rates for colon, breast, and prostate cancer.

During the past 20 years, research on the biochemical nature of cancer cells and the responses of normal organs and tissues to cancer has advanced dramatically, and a detailed molecular understanding of these processes is emerging. This knowledge will serve as an increasing part of the foundation of future care and prevention.

In the past 25 years, research has elucidated molecular mechanisms directing key aspects of tumor cell behavior. Detailed understanding of these mechanisms has already changed methods for diagnosis, prognosis, and treatment. With continuing advances in cancer science and the emergence of new technologies for applying basic science to clinical practice, new methods based on molecular mechanisms will dominate cancer care and prevention.

New Advances in Cancer Science
Cancer is, in significant measure, a disease due to acquired or inherited misbehavior of genes that involve nearly all of the known signal transduction pathways of cells. These pathways influence cellular economy, including proliferation, survival, threat avoidance, energy utilization, and adhesion to other cells and to membranes, as well as embryonic pathways normally engaged for the development of organs and the operations of certain organ-specific functions. Understanding the molecular nature of specific signal transduction events has enriched current cancer research immeasurably.

Signal Transduction in Normal Cells and Their Malignant Derivatives. Understanding of the molecular basis for carcinogenesis has changed radically during the past 50 years. For example, elucidation of detailed biochemical controls governing the cellular division cycle has demonstrated that most human tumors have defects in a particular signal transduction pathway, the retinoblastoma (RB) pathway (FIGURE 1). The RB pathway normally regulates the ability of cells to exit from the resting phases (G0 and G1) of the cycle and to proliferate.

The product of the retinoblastoma susceptibility gene (pRB) is a complex protein that partly operates to repress the transcription of certain genes that encode proteins that promote cell cycle progression.1 P16INK4a is a specific inhibitor of the cyclin D-dependent protein kinases (cdk4 and 6) that normally phosphorylate pRB. Phosphorylation of pRB in this setting inhibits the binding of certain transcriptional repressor proteins and members of the E2F transcription factor family. Interactions of pRB with these proteins are essential for its transcriptional repression and G1 exit-suppression function. After phosphorylation of pRB, cell cycle progression genes that were formerly repressed are activated, with ensuing cell proliferation.

Thus, mutations that eliminate p16 synthesis, render cdk4 and 6 unable to be inhibited by p16, increase the abundance of cyclin D1, render pRB nonfunctional, or perturb the function of the
The Hypoxia-Inducible Factor Pathway of Hypoxia Control

**Figure 2. The Hypoxia-Inducible Factor Pathway of Hypoxia Control**

- **A** Normoxic Conditions: Degradation of HIF-1α
  - VHL recognizes HIF-1α and ubiquitinates it, leading to its proteolytic degradation.

- **B** Hypoxic Conditions: Stabilization of HIF-1α
  - Hypoxia stabilizes HIF-1α, which forms a complex with ARNT and activates the transcription of genes involved in angiogenesis, erythropoietin synthesis, and anaerobic glycolysis.

**Figure 1. The Retinoblastoma Pathway of Cell Proliferation Control**

- The **RB pathway** can all promote unregulated entry into S phase. Indeed, most human tumor cell types display a defect in the RB pathway. These observations represent major progress in understanding why most tumor cells have impaired control of proliferation.

- **Apoptosis.** Research on cellular behavior has also led to the recognition that cells carry a lifespan control program, one outcome of which is rapid cell death by a process known as apoptosis. For example, the thymus eliminates many developing T cells, most of which have autoimmune potential, by promoting their controlled apoptosis. During development, organ size is regulated, in part, by tight controls on the numbers of cells in that organ, including death by apoptosis of the excess cells.

- Many human tumor cells should have died long before their emergence as components of a tumor mass, but these cells have acquired molecular mechanisms that confer resistance to apoptosis. The first indication of such a tumor-associated apoptosis-resistance mechanism was detected during an analysis of the functional significance of a translocation in follicular lymphoma that links an immunoglobulin promoter to a gene called Bcl2. The product of this gene physically interacts with and inhibits the action of 1 or more structurally related proteins (eg, Bax) that promote apoptotic activity. The molecular abnormalities that prevent apoptosis of some human tumor cells result from defects in a signal transduction pathway, key elements of which were first deciphered in the nematode *Caenorhabditis elegans* by Horvitz and coworkers. Thus, components of this pathway have been preserved during evolution, pointing to its importance in...
the control of body pattern and organ development.

Angiogenesis. Studies of angiogenesis, begun 25 years ago by Folkman and now pursued further, have provided the first explanations of why tumor cells acquire the capability to invade and metastasize during proliferation.8 The ability of tumor cells to induce endothelial cells to proliferate, form small vessels, and infiltrate tumor cell–containing tissue is fundamental to tumor growth and invasion.

The molecular signals that emanate from tumor cells and induce vessel development include specific polypeptide factors, such as vascular endothelial cell growth factor (VEGF) and basic fibroblast growth factor, which have the potential to engage endothelial cells and either promote or suppress their proliferation and survival. These factors play a major role in tumor angiogenesis.9 In 1 pathway, the von Hippel Lindau (VHL) tumor suppressor gene product (loss of function of which underlies VHL disease and contributes to about 70% of renal cell carcinoma) normally promotes degradation of a transcription factor called HIF (hypoxia-inducible factor)10 (FIGURE 2). Hypoxia-inducible factor is stabilized by localized tumor-associated hypoxia, a common accompaniment of tumor growth. When HIF is stabilized, multiple genes are activated, including that for VEGF, which results in endothelial cell proliferation, migration of epithelial cells toward the anoxic tissue, and formation of capillaries that eventually supply the tumor bed. Renal cell carcinomas are often highly vascularized, and lack of VHL function contributing to failure of HIF degradation likely contributes to this characteristic.

Insight into the steps in the tumor angiogenesis process made it possible to develop a variety of experimental perturbants. These include inhibitors of the tyrosine kinase function of at least 1 species of VEGF receptor, a monoclonal antibody to this receptor, and 1 naturally occurring polypeptide (endostatin)8 with antiangiogenic and antitumor activity in experimental systems.

Cancer and Development. Recent insights imply that tumors are actually small organs with many of the nutritional and other features of their normal organ counterparts. The most striking insight is that individual tumor cells, and presumably the tissue masses composed of them, develop under the influence of mutant genes which, in their wild type (wt) state, normally promote organ development during embryogenesis. For example, the sonic hedgehog and patched genes normally promote the development of a number of organs, including parts of the brain.11 However, mutant forms of one of these genes in the germ line can cause development of medulloblastoma or basal cell nevus syndrome.12

Similarly, the Wnt signal-transduction pathway is vital for several types of organogenesis, including those leading to formation of the intestinal epithelium13 and the breast.14 Mutation of either of 2 participants in the Wnt pathway (adenomatous polyposis coli [APC] and b-catenin) can promote the development of colon cancer, both the inherited and the sporadic type. Since mutant species of organ-forming genes participate in neoplasia, it seems reasonable to hypothesize that their by-products (ie, tumors) may have arisen by abnormal processes which, in their normal state, would have led to proper organ homeostasis.

Cancer Genes. No discipline has provided more insight into the abnormal behavior of cancer cells than genetics, and a myriad of oncogenes and tumor-suppressing loci are now known. Small numbers of tumor cells can be dissected from surrounding normal tissue15 and their complement of oncogenes and defective tumor-suppressing genes can be defined by molecular genotyping methods, usually involving the polymerase chain reaction. Patterns of oncogene and defective tumor-suppressor participation have been identified in many common and less prevalent tumors (eg, the genetic events that contribute to sporadic colon cancer16), and in some instances correlations have been made with clinical behavior. The first of these associations was the finding of the 9:22 translocation in chronic myelogenous leukemia (CML) by Nowell et al.17 This translocation gives rise to the bcr-abl fusion gene and protein, a tyrosine kinase, the protein phosphorylating function of which contributes to the survival and neoplastic behavior of CML tumor cells.18

Cells of many human tumors—particularly those of epithelial origin—exhibit defects in their ability to maintain a stable genome, such as excess or abnormally formed chromosomes, or both. Furthermore, some human tumor-suppressor genes (eg, p53, certain mismatch repair genes, and BRCA1 and BRCA2) are involved, at least in part, in maintenance of genome integrity, including control of chromosome number. Cancer cells may gain advantage from the loss of controls of genome stability, because in this state they can more readily acquire new mutations, some of which have the potential to enhance neoplastic power.

It is now possible to assess gene structure and gene expression and to categorize individual human tumors with respect to the molecular mechanisms that sustain them. This practice has led to an ability to estimate prognosis for patients with the same disorder (eg, large B-cell lymphoma and acute leukemia), some of whom have much better prognoses than others.19,20

The sequencing of the entire human genome and, in the future, of all human messenger RNA, will make it possible to probe the expression of all human genes in a given tumor cell. For example, it is now possible to characterize acute leukemia cells with respect to the cell of origin (lymphoid vs myeloid) based on this approach.21

Future Clinical Applications
Tools that facilitate analysis of individual cancer cells and tumor masses now exist, and are being applied to the analysis of patients with cancer. Technology is also being developed to facilitate biological, biochemical, and genetic analysis of small numbers of human cancer cells. This activity, and
the fact that more is known of the molecular bases for tumor behavior, suggests that many of the remaining mysteries of high importance in oncology will be solved in a reasonable period of time. What clinical benefits can be expected from research on tumor mechanisms?

**Mechanism-Based Diagnosis and Prognosis.** Clinical oncology largely depends on the recognition of tumor deposits by anatomy-based methods. In addition to direct visualization of tumors, cytological examination of fluids, and pathological analyses of biopsy materials, sophisticated radiographic and nuclear scanning methods are now standard tools for cancer diagnosis. In addition, a variety of immunological and cell biology/biochemistry-based cell-staining methods are available for denoting the organ and cell of origin and the clinical subspecies of individual tumors. Less commonly used are cytogenetic or molecular genetic methods, except where a specific chromosomal abnormality is a hallmark of a given tumor (eg, CML, acute promyelocytic leukemia, synovial sarcoma, acute myelogenous leukemia). In essence, success in diagnosis depends on the presence of sufficient numbers of tumor cells to be recognized anatomically. In this context, computed tomographic methods and magnetic resonance imaging have greatly increased the degree of anatomical detail that can be probed, thereby enhancing the accuracy with which tumors can be detected. Lesions once undetectable, even at surgery, are now commonly recognized without resorting to invasive or operative procedures.

Nevertheless, the lower limits of recognition size for most lesions are in the range of hundreds of millions to billions of cells. This means that the early history of most tumors goes unrecorded, because there is no practical way of detecting them before they reach the minimal radiographic (or pathological) detection limit. There are exceptions, such as in situ cervical and some skin cancers, but their number is not large.

In many tumors, the lower limit of recognition size is frequently too great to allow diagnosis before development of metastatic disease. For certain epithelial tumors, an expanding tumor mass accumulates cells that have sustained a spectrum of genetic abnormalities. When the numbers of cells with the right properties to elicit more invasive behavior accumulate, the tumor becomes a serious clinical threat. Therefore, it would be beneficial to recognize tumor masses when they are much smaller than the current clinical recognition limit.

In this regard, better understanding of the molecular mechanisms of invasion and metastasis and better technology for the genetic characterization of individual tumor cells will be coupled with new imaging methodologies (including optical methods and nanotechnologies) capable of detecting many fewer tumor cells than is now possible. Such advances may also make possible the development of detection methods to characterize small numbers of tumor cells with respect to their invasive and/or metastatic potential. As knowledge of defective signal transduction behavior in tumor cells has increased, so has the capability to recognize tens of thousands, as opposed to millions, of tumor cells in animals. In one approach, detection of a few thousand subcutaneously implanted tumor cells, each expressing a firefly luciferase gene, is based on an ability to detect a luciferase-catalyzed light signal emitted through the skin. It would be ideal to be able to characterize tumor cells as to whether they are capable of performing certain functions that their normal counterparts cannot perform. These functions need not only be enzymatic, but might reflect abnormal interactions of one gene product with another, eg, the binding of the apoptosis-inhibiting protein Bcl2 to the apoptosis-promoting protein Bax, an interaction characteristic of disorders such as follicular lymphoma and prostate cancer.

Another possibility is detecting a Bcl2-producing cell by imaging techniques, such as using small molecules that can selectively recognize, with high affinity, abnormal structural features of pro-

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teins in cancer cells. These compounds can become lead compounds necessary for the discovery and development of new antitumor agents, and they can also be modified to bind a targeted, cancer cell–specific protein structural element (eg, Bcl2/Bax) and to emit a physical signal (eg, light, radioactivity). If the signals are powerful enough and the detection systems sufficiently sensitive, this approach might be useful for detecting small numbers of tumor cells in a noninvasive manner, much as is the case for the luciferase-based method described above.

With the availability of supersensitive methods of detection, it should become possible to define the lower limits of detection for each small-molecule compound in patients with cancer. Recognition of subclinical tumor deposits by super-sensitive tumor-cell imaging methods could stimulate lines of research not presently feasible. For instance, the prospect of being able to recognize tens or hundreds of thousands—as opposed to a billion or more—lung cancer cells in heavy smokers would have dramatic implications for research on cancer therapies.

Prospects for Mechanism-Based Therapeutics. Rational cancer drug development based on disease mechanisms is now a highly active field. Mechanism-based treatments include those based on absolute biochemical differences between tumor cells and their untransformed counterparts, such as the presence of the bcr-abl tyrosine kinase in CML cells and its absence in normal bone marrow. A new, highly specific tyrosine kinase inhibitor, STI571, can induce complete remissions in some otherwise drug-resistant patients with minimal toxicity. This agent, still in clinical trials, is a new paradigm for targeted cancer drug development.

Another example is the selective presence of the PML-RAR (retinoic acid receptor) fusion protein in acute promyelocytic leukemia (APML) cells. In this case, many patients experience complete remission after treatment with all-trans-retinoic acid, a generally innocuous, natural product that binds with high affinity to the retinoic acid binding domain of the fusion protein. Binding of this ligand selectively eliminates APML leukemic cells with minimal toxicity, most likely by inducing apoptosis.

Analogous strategies will likely be applied for other tumors. Current cancer research is aimed, in part, at deciphering which proteins potentially contribute to the aggressive behavior of tumors and how they do so. For example, in most acute leukemias, a specific chromosomal translocation results in the synthesis of fusion proteins. More than 30 such fusion genes have been identified and are major contributors to the malignant effects of the offending cells. Most of these fusion proteins are transcription factors. These proteins have traditionally represented a challenge to those working on identifying small-molecule perturbants of their function. However, it is likely that a number of the leukemia cell fusion proteins will in time become useful targets for small-molecule drug discovery.

Mutant gene products also operate in certain common solid tumors. The tumor suppressor gene p53 is mutated in almost half of all solid tumors, and the mutant allele usually encodes a protein that interacts with the product of the wt locus and down-modulates its normal function. As a consequence, cells behave as if they had lost most normal p53 function, a feature that contributes to the malignant phenotype of many tumor cells. The p53 protein is also a transcription factor, and at least 1 small-molecule compound that interacts with a specific domain of a p53 mutant protein can induce a conformational change that effectively makes the protein behave like its normal counterpart. Most importantly, the small-molecule-bound protein regains tumor suppressing activity. Analogous efforts will be aimed at redirecting the biochemical behavior of proteins shown by genetic, cell biological, and/or biochemical means as contributing to the neoplastic state of other tumors. This line of research, or a derivative one, holds promise for the development of new, clinically effective agents.

The structural domains to be targeted need not be small-molecule binding units (eg, active sites of enzymes, hormone-binding domains of receptors). Identifying other ordered types of polypeptide and even nucleic acid structures could greatly expand the repertoires of targets available for cancer drug discovery. This is because many of the molecular mechanisms of cancer cells depend on the abnormal regulatory behavior of 1 or more signal transduction proteins, many of which normally lack dedicated small-molecule binding domains.

“Smart” drug screens of the type that led to the mutant p53 antagonist will become more frequent as the bases for the tumor-suppressing behaviors of individual proteins such as p53, pRB, p16 INK4A, VHL, APC, and others are better understood. In time, it may be possible to identify specific compounds capable of inducing the activity of mutant Ras proteins to behave normally. Since constitutively activated Ras protein often contributes to the neoplastic state of some of the most common human tumors (eg, colon, lung, pancreas), an ability to down-modulate its oncogenic function is an appealing prospect.

This goal is also a major challenge, because the chemical differences between active and inactive Ras are limited and subtle. However, the ability to identify products capable of perturbing defined oncprotein targets is improving and the drug discovery process is likely to produce compounds with ever more specific antitumor effects. Indeed, a number of compounds that perturb key cancer-abetting proteins are in clinical trials, and more are expected. Since these agents perturb specific participants in the neoplastic process, adverse effects should be less serious and widespread (as in the cases of STI 571 and all-trans-retinoic acid).

Future candidate drugs should derive from knowledge of gene products that participate in the aggressive behavior of a given tumor. Analysis of detailed tumor genotype, gene expres-
sion phenotype, and selected biological characteristics could predict the outcome of treatment with a given compound or combination of compounds. This is already the case for breast cancers that do or do not synthesize measurable quantities of the estrogen receptor (influencing susceptibility to tamoxifen) and HER-2 neu (influencing susceptibility to trastuzumab) and for acute leukemia cells that synthesize PML-RAR (influencing susceptibility to all-trans-retinoic acid). The results of molecular tumor analysis will make possible ever more accurate predictions of which strategies are applicable to a given tumor, and empirical choices of treatment are likely to be replaced by rational ones. Moreover, by sampling tumor cells during failed treatment, it may also be possible to change therapy as key elements of the molecular phenotype improve tumor response.

New molecular imaging methods that reflect biochemical outputs of perturbing a given drug target are also possible. In a tumor mass, for example, it might be possible to image the key biochemical effects of delivering a specific inhibitor/perturbant of a given protein target. The presence of an imageable effect would mean that a drug of interest had reached, bound, and perturbed the desired target. An ensuing antitumor response would be a gratifying accompaniment to such an effect, and its absence would mean that a resistance mechanism was present but did not result from failure of the agent to perturb the desired molecular target.

Finally, understanding the molecular basis for cellular immunology has also progressed to a point where it is possible to induce immunity against certain epithelial tumor cells. Many potential tumor vaccines are now in (or entering) clinical trials, and efforts to induce specific tumor immunity by mechanism-based methods will continue.

Conclusion

As the understanding of molecular cancer biology progressively influences both clinical tumor characterization and treatment strategy, better therapeutic outcomes can be expected. However, many technological hurdles must be overcome before the design of rational treatment can enter daily clinical practice. The body of clinically relevant cancer biology has reached a point of critical mass, setting an unalterable course toward an attack on tumor cells based on what made these cells different in the first place.

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REFERENCES