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Cancer—Emerging Breakthrough Drugs

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Cancer may soon become the leading disease-related cause of human death in many countries of the world. In the United States, cancer is projected to surpass cardiovascular disease as the major cause of death. Nevertheless, there is good news forthcoming with respect to emerging breakthrough drugs that demonstrate our increased understanding of cancer at the molecular level, a greater focus on mechanism-based target identification, and the use of sophisticated genetic, cellular, and animal pharmacological models to more comprehensively evaluate potential cancer drugs. We anticipate that our knowledge of the human genome and the genetic basis of disease, as well as the integration of drug discovery and genomic technologies, will help us to create new treatments for diseases such as cancer that will be effective, safe, and ultimately provide cures. This column addresses the critical elements and scientific approaches that underlie the “genes to drugs” campaign to discover promising new classes of cancer therapeutics.

Unraveling the Complexity of Cancer at the Molecular Level

Cancer can involve dynamic changes in the genome. Many investigations have shown that a wide variety of cancer cells have altered forms or expression levels of proteins that are intimately involved in signal transduction pathways (4,5). Examples of such proteins include certain protein kinases, G proteins, transcription factors, nuclear receptors, and growth factor receptors. The genes ultimately responsible for these altered proteins are known as oncogenes. Some examples of oncogenes (and their gene products) include *src* (Src protein kinase), *abl* (Abl protein kinase), *raf* (Raf protein kinase), *gsp* (G protein α -subunit), *jun* (transcription factor [AP-1]), *fos* (transcription factor [AP-1]), *myc* (DNA-binding protein), *erbA* (thyroid hormone receptor), *erbB* (epidermal growth factor receptor), *trk* (nerve growth factor receptor), *ros* (insulin receptor), and *kit* (platelet-derived growth factor receptor).

The first discovered cancer-causing retrovirus was Rous sarcoma virus. Subsequent investigations led to the identification of the viral oncogene *src* and its gene product Src, a protein tyrosine kinase, as the transforming element. Comparative analysis of viral Src (v-Src) and cellular Src (c-Src) have shown differences in their amino acid sequences, and both biochemical and 3-D structural studies have provided a basis to explain the loss of self-

regulatory properties observed for v-Src. In normal cells, *src* is a proto-oncogene. Elevated c-Src protein levels and/or its tyrosine kinase activity have been identified in some cancers such as colon, breast, pancreatic, and lung. Interestingly, a *src* gene knockout in mice results in a phenotype exhibiting osteopetrosis (excessive bone formation). It is important to point out that Src was the first of the superfamily of protein tyrosine kinases to be identified and characterized. Its structure includes two non-catalytic domains (SH3 and SH2), which have been found in other diverse signal transduction proteins, and a tyrosine kinase catalytic domain.

A second milestone in cancer research was the isolation and analysis of activated oncogenes from human tumors. This ultimately led to the discovery of the *ras* proto-oncogenes and a family of Ras proteins that possess GTPase activity similar to the α -subunit of G proteins. Both biochemical and 3-D structural studies have provided insights for understanding how Ras proteins function in signal transduction pathways between growth factor receptors and their activation of specific genes. Mutations of Ras proteins have been identified in about 25% of human tumors (nearly 50% of human colon cancers) and affect mitogenic signaling without upstream regulatory stimulation.

A third significant type of genetic alteration found in cancers involves tumor suppressor genes. In contrast to the oncogenes, deficient tumor suppression has been correlated with some cancers. Examples of well-known tumor suppressor genes are those that encode retinoblastoma protein (Rb) and p53 protein. Mutations of the retinoblastoma gene are known to cause a type of eye tumor. Unregulated pathways that otherwise signal through Rb as a checkpoint for cell cycle progression from G1 into S phase can result in uncontrolled cell proliferation. Mutations of p53 proteins have been identified in about 50% of human cancers. Functionally, p53 is a DNA-binding protein that plays a pivotal role in regulating the cell cycle, preventing inappropriate movement of cells from G1 to the S phase). Hence, a loss of p53 function as a checkpoint would lead to uncontrolled cell growth.

Beyond such representative examples that have helped to unravel the complexity of cancer at the molecular level, it is imperative to point out that human cancers contain a series of mutations of different genes and their gene products. In fact, there are more than 100 distinct types of cancers (some with additional subtypes in specific organs). Not surprisingly, many details remain to be determined in the identification of genes and gene products that underlie the molecular pathogenesis for many forms of cancer.

The transformation of normal cells to full-fledged tumor cells involves an accumulation of acquired functional capabilities: (i) self-sufficiency in growth signals, (ii) insensitivity to antigrowth signals, (iii) unlimited replicative potential, (iv) evasion of apoptotic signals, (v) tissue invasion and metastasis, and (vi) sustained angiogenesis. Therefore, it is not unexpected that identification of therapeutic targets will ultimately correlate with one or more of such acquired functional capabilities of cancer cells.

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Identifying Therapeutic Targets for Cancer Drug Discovery

In retrospect, some of the first cancer drugs have been effective at the molecular level by modifying DNA (e.g., alkylating agents) to impair replication or to inhibit key enzymatic steps in nucleotide biosynthesis (e.g., methotrexate). Currently, a major shift toward therapeutic targets involved in signal transduction pathways, cell cycle regulation, apoptosis, and angiogenesis has become the focus of cancer drug discovery (1–3,6,9). Protein kinases account for the majority of such therapeutic targets, including examples that are compartmentalized at the cell membrane, in the cytoplasm, and in the nucleus. The most widely recognized among these protein kinases for cancer drug discovery are the EGF-R, FGF-R, PDGF-R, vascular endothelial growth factor receptor (VEGF-R/KDR), Bcr-Abl, Src, PKC, and cyclin-dependent kinases (CDKs). In the case of the Ras signal transduction pathway, mutant Ras proteins have proven to be a difficult target for drug discovery. However, two key enzymes in the Ras signaling pathway, Raf and MEK (both protein kinases), have been identified as promising therapeutic targets.

In the cell nucleus, specific CDKs are negatively regulated by p16 protein. For some cancers with defective p16 protein, it is believed that such CDKs represent compelling therapeutic targets for drug discovery.

As a paradigm for cancer research, breast cancer provides several therapeutic targets that have led to the development of both cytotoxic- and mechanism-based drugs. These drugs include doxorubicin (Adriamycin), an inhibitor of topoisomerase function, and paclitaxel (Taxol), an inhibitor of tubulin polymerization. In the latter case, tumor cell apoptosis is induced by a novel G2/M checkpoint that is independent of p53 function. Estrogen receptor antagonists such as tamoxifen (Nolvadex) have shown success in human breast cancers that are hormone dependent. Current drug discovery efforts are focused on specific estrogen receptor subtypes that may make possible selective estrogen receptor modulators (SERMs) that possess higher efficacy with minimal side effects. The HER-2 (erbB2) receptor is commonly overexpressed in human breast cancers, and the first successful therapeutic strategy that focused on a human oncogene was devel-

Figure 1. Some known signal transduction and regulatory pathways in cells. Not all possibilities and variations are shown.

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oped against the HER-2 receptor. Specifically, the humanized monoclonal antibody trastuzumab (Herceptin), which binds to the extracellular domain of the HER-2 receptor, has been approved by the FDA for the treatment of HER-2-positive breast cancers (accounting for about 30% of breast cancers).

The complexity of signal transduction and regulatory pathways in cells illustrates the challenge of identifying therapeutic targets for cancer drug discovery. Many proteins have been found that control cell function, proliferation, and survival. Although oversimplified, the depiction of selected signal transduction pathways in Figure 1 provides a basis for conceptualizing both the discrete nature of intracellular pathways and the possibilities for crosstalk. Furthermore, and taking into account the contributions of ancillary cells present in a tumor (e.g., fibroblasts, immune and endothelial cells), surmounting evidence implicates that a consortium of heterotypic cell-to-cell communications is critically involved in the pathogenesis of many cancers. Elegant and comprehensive reviews of this subject have been recently published (4,5).

New Small-Molecule and Peptidomimetic Drugs

A tremendous drug discovery effort in industry, government, and academic research laboratories has advanced new cancer therapeutics, including small-molecule and peptidomimetic drugs, recombinant proteins, vaccines, monoclonal antibodies, antisense RNA, ribozymes, and DNA (gene therapy). It would be impossible to adequately discuss such a wide scope of cancer therapeutics in this column; however, recent excitement has surged with respect to a number of emerging breakthrough drugs that exemplify the potential success of small-molecule and peptidomimetic inhibitors of key signal transduction pathways known to be unregulated in cancer cells (1–3,7,9).

A number of promising inhibitors of protein kinases have been discovered and advanced into animal models and/or clinical testing. A vast majority of such examples have been designed to inhibit ATP-binding at the active site of the catalytic domain of the target protein kinase. The success of this strategy has been based on the concept that there are discrete differences (e.g., amino acid sequence variations as well as conformational differences between inactive and active forms of the enzyme) at such ATP binding pockets. Lead compound identification and optimization efforts have been further supported by kinase-targeted screening and structure-based design (X-ray- and/or homology model-based) approaches. A number of small-molecule inhibitors varying in the core template have been developed to replace the adenine ring substructure of ATP. Such core templates include quinoxalines, various types of heterocyclic fused-pyrimidines, phenylamino-pyrimidines, and indolinones. The 3-D structures of several ATP-related inhibitors complexed with their target protein kinase have also influenced the structure-based drug design of second-generation lead compounds. Currently, there are more than 60 protein ki-

nase 3-D structures in the Protein Data Bank, including some ligand-enzyme complexes.

Among growth factor receptor tyrosine kinase therapeutic targets, drug discovery research on EGF-R and VEGF-R (KDR) tyrosine kinase inhibitors is noteworthy. A number of EGF-R tyrosine kinase inhibitors have already progressed to human clinical testing (e.g., ZD-1839, OSI-774, and PD-183805). These compounds generally exhibit significant specificity for EGF-R tyrosine kinase, potent cellular activity, and in vivo efficacy in animal models (e.g., inhibition of the growth of tumor xenographs). One EGF-R tyrosine kinase inhibitor (ZD-1839) has been assessed in human clinical trials and has shown promising antitumor activity against non-small cell lung cancer and head/neck cancer. A VEGF-R kinase inhibitor (SU-5416) is in human clinical testing to evaluate its inhibition of tumor angiogenesis. It is a potent and selective inhibitor of VEGF-stimulated mitogenesis in human endothelial cells.

For intracellular nonreceptor tyrosine kinase therapeutic targets, drug discovery efforts on Bcr-Abl, CDKs, and Src are also noteworthy. A Bcr-Abl tyrosine kinase inhibitor (STI-571) is in human clinical testing for CML, and the results have been exceptional to date. A CDK inhibitor (Flavopiridol) is currently in human clinical trials for evaluation against refractory myeloma, advanced human gastric carcinoma, and several other conditions. Recently developed Src tyrosine kinase inhibitors (PD-180970 and CGP-77675) have been found to effect antiproliferative activities in several colon cancer cell lines.

Similar to the tremendous strides in cancer drug discovery for protein kinase inhibitors, significant efforts have focused on the development of farnesyltransferase (FTase) inhibitors. Several FTase inhibitors have recently entered human clinical testing (e.g., R-115777, SCH-66336, and ABT-839). The concept for the discovery of FTase inhibitors was that mutant Ras signaling blockade might be achieved by interrupting its post-translational modification by FTase, thereby resulting in a functionally inactive form of the enzyme. In fact, earlier studies showed that mutant Ras proteins had to be farnesylated to influence cell proliferation. A variety of FTase substrate-based, peptidomimetic inhibitors and second-generation nonpeptide inhibitors (from FTase-targeted screening and/or structure-based design) have been created. The 3-D structures of a few FTase-inhibitor complexes have also been recently determined.

However, the most significant finding from studies with FTase inhibitors has been the lack of consistent correlation of their cellular properties with Ras-positive versus Ras-negative human tumor cells. However, because of the antiproliferative activity of a number of FTase inhibitors, the question has now become one of identifying likely therapeutic targets other than Ras. Interestingly, it has been determined that more than 300 candidate proteins have the Cys-containing peptide sequence characteristic of substrates (e.g., Ras protein) for FTase. Furthermore, it is known that HMG-CoA reductase inhi-

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bitors (i.e., the statins) and nitrogen-containing bisphosphonate drugs function, at least in part, by thwarting protein prenylation by inhibiting enzymes critical to the biosynthesis of farnesyl-diphosphate. Hence, the FTase inhibitors remain promising as cancer therapeutics and have guided new directions to other therapeutic targets and diseases.

Other examples of emerging small-molecule and peptidomimetic cancer drugs include SERMs, matrix metalloproteinase (MMP) inhibitors, and drugs targeting a host of other intracellular enzymes and protein-protein interactions that are critically involved in unregulated cell growth, apoptosis (e.g., Bcl-2 and caspases), and replication (e.g., telomerases). Furthermore, drug discovery efforts are making inroads to more complex homotypic and heterotypic cellular processes related to angiogenesis (e.g., VEGF-R, FGF-R, and integrins) and tissue invasion and metastasis (e.g., E-cadherin and integrins). Metastases are the cause of a majority (estimated at 90%) of human cancer deaths.

Winning the War against Cancer

The war against cancer may be won one battle at a time. As the therapeutic targets become better understood

and the molecular mechanisms continue to be clarified, it will be clearer where to target both our existing and emerging cancer drug arsenal. The wide range of therapeutic modalities exceeds by far the scope of small-molecule and peptidomimetic drugs discussed in this column. Recombinant proteins, vaccines, monoclonal antibodies, antisense RNA, ribozymes, and DNA (gene therapy) have also achieved varying degrees of success. The casualties have been, regrettably, extraordinarily high in the war against cancer.

Understanding genes and gene products will undoubtedly illuminate the complexities of cancer at the molecular level. Knowledge of the human genome sequence and its most currently estimated 35 000 genes will serve tremendously in such endeavors (6,10). Efforts to further understand the molecular pharmacology of human cancer include the use of cDNA microarray technologies in recent studies at the National Cancer Institute (8). This work has focused on the determination of gene expression profiles for a significant number of human cancer cell lines that have been previously subjected to screening by more than 70 000 potential cancer drugs. Such a gene expression database may provide a major step toward selecting therapeutic modalities based on the molecular characteristics of a patient's own cancer.

REFERENCES

1. Dalgarno, D.C., C.A. Metcalf III, W.C. Shakespeare, and T.K. Sawyer. 2000. Signal transduction drug discovery: targets, mechanisms and structure-based design. *Curr. Opin. Drug Disc. Dev.* 3:549-564.
2. Garrett, M.D. and P. Workman. 1999. Discovering novel chemotherapeutic drugs for the third millennium. *Eur. J. Cancer* 35:2010-2030.
3. Gibbs, J.B. 2000. Mechanism-based target identification and drug discovery in cancer research. *Science* 287:1969-1973.
4. Hanahan, D. and R.A. Weinberg. 2000. The hallmarks of cancer. *Cell* 100:57-70.
5. Hunter, T. 2000. Signalling—2000 and beyond. *Cell* 100:113-127.
6. International Human Genome Sequencing Consortium. 2001. Initial sequencing and analysis of the human genome. *Nature* 409:860-921.
7. Kelloff, G.J. 2000. Perspectives on cancer chemoprevention research and drug development. *Advances Cancer Res.* 78:199-334.
8. Scherf, U., D.T. Ross, M. Waltham, L.H. Smith, J.K. Lee, L. Tanabe, K.W. Kohn et al. 2000. A gene expression database for the molecular pharmacology of cancer. *Nat. Genet.* 24:236-244.
9. Sedlacek, H.H. 2000. Kinase inhibitors in cancer therapy. *Drugs* 59:435-476.
10. Venter, J.C., M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G. Sutton, H.O. Smith, et al. 2001. The sequence of the human genome. *Science* 291:1304-1351.

Suggestions for contributions to the "Drug Discovery and Genomic Technologies" section are welcomed by its editor, Dr. Tomi K. Sawyer (tomi.sawyer@ariad.com)