Southern Medical Journal

Volume 96(12), December 2003, pp 1174-1186

Biotechnology and Drug Discovery: From Bench to Bedside

[Original Articles: Original Article]

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Accepted January 21, 2003.

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Each of us contributed equally to this article. We have no commercial, proprietary, or financial interest in any drug, device, or equipment mentioned in this article.

Abstract

New biotechnology and drug discovery technologies are facilitating the rapid expansion of the clinical drug chest, empowering clinicians with a better understanding of disease as well as novel modalities for treating patients. Important research tools and themes include genomics, proteomics, ligand-receptor interaction, signal transduction, rational drug design, biochips, and microarrays. Emerging drug classes include monoclonal antibodies, cancer vaccines, gene therapy, antisense strands, enzymes, and proteins. In this article, we review these topics and illustrate their potential impact by presenting an overview of promising drugs in the pipeline. Clinicians who use these novel treatments must become familiar with these trends.

Biotechnology is introducing new capabilities to drug discovery, which were considered until recently to be impractical and futuristic. There has been continuous evolution in the integrated approach to the development of therapies in medicine. This effort relies on clinicians, basic scientists, and feedback from novel translational applications.

Initially, biotechnology was synonymous with the emerging recombinant deoxyribonucleic acid (DNA) technology and was used for the large-scale production of proteins, initially "replacement" proteins such as insulin and factor VIII. Later, developments were based on an understanding of ligand-receptor interactions, their impact on disease processes, and the ability to manufacture such large macromolecular proteins for therapeutic purposes. Today, signal transduction and

cell signaling and their role in normal and disease states are taking center stage. Small-molecule drug (SMD) discovery, which uses and builds on organic molecules as starting materials, is also benefiting from the input of newer technologies such as combinatorial chemistry and high-throughput screening.

Although many physicians are not exposed to biotechnology, we think that it is valuable for clinicians to gain some fluency in the important trends in this field because the fruits of biotechnological research are reaching the clinic. The speed of events that are occurring in biotechnology is breathtaking and inspiring indeed. The younger generation of physicians has had the privilege of studying molecular biology as medical students. Even those physicians who did study molecular biology in medical school, however, must be excited but somewhat bewildered and uncomfortable about the advent of novel treatment modalities involving the use of antisense strands and monoclonal antibodies (MAb). This review is aimed at practitioners and specialists who are not closely involved in the process of drug discovery and intends to highlight the main developments in biotechnology and their impact on medicine.

Overview of Biotechnology

Drug discovery and development are costly and complicated processes. More than 99% of experimental compounds ultimately fail or are discarded as Treatment regimens. Of the chemicals evaluated as part of drug discovery and preclinical testing, only a few proceed to human clinical trials and are approved for marketing. 1 To address this issue, new therapeutic approaches based on genomic and proteomic technology have been developed during the past several years. The -omic suffix is an example of the lexicon that has emerged to define the varied populations and subpopulations in the cell. These terms generally carry the –ome suffix, with an associated research topic denoted by the -omics appellation. The "genome"-that is, the full complement of an organism's genetic information that includes both coding and noncoding DNA sequences-provides a basis for defining the "proteome," which is a list of only the encoding DNA regions that result in protein products. 2 Genomics and proteomics enable the discovery of new genes and proteins and the comparison of their levels in diseased cells, normal cells, and cells treated with compounds that vary in their efficacy and toxicity. Thus, they could prove valuable in identifying new drug targets.

Key Points

- As a result of new biotechnological capabilities, the understanding of disease processes and the development of new treatments are expanding rapidly.

- Important tools and developments include genomics, proteomics, ligand-receptor interaction, signal transduction, rational drug design, biochips, and microarrays.

- There are several novel drug classes, each with its own structural architecture and mechanism of action, including monoclonal antibodies, cancer vaccines, gene therapy, antisense strands, enzymes, and proteins.

- A wealth of promising new drugs will enable better treatments for patients with cancer, autoimmune disease, neurologic disease, allergy, and transplant rejection, among other entities.

In drug discovery, the drug target is key. A target for pharmaceutical intervention is almost invariably a protein whose function or dysfunction is implicated in a disease process-for instance, growth factors and their receptors, which are frequently overexpressed in carcinomas. 3 A case in point is the epidermal growth factor (EGF) receptor family, which is the most studied growth factor receptor system. These receptors are composed of an extracellular binding domain, a transmembranous lipophilic segment, and an intracellular protein tyrosine kinase domain with a regulatory segment. The interaction of the extracellular growth factor with its receptor (ie, ligand-receptor interaction) results in the activation of cell signaling pathways that lead ultimately to cell division, the synthesis of new proteins, and tumor progression. This cascade of events is known as signal transduction. Figure 1 illustrates the different steps along the EGF signal transduction pathway that can serve as targets, some of which are being addressed already.

Manifold new drug targets are expected to sprout from the Human Genome Project, which has focused much attention on biotechnology. Indeed, the insight that the Human Genome Project provides with regard to the cell's genetic makeup as well as disease states can be used to understand the cell's protein makeup to generate new protein targets for intervention, such as the EGF family. However, the gene-protein-disease triangle is complex. Therefore, additional contributions from genomic and proteomic technologies is necessary to understand the genetic makeup and expression of diseased cells as well as how the resulting cellular proteins interact to cause disease. For example, biochips are one key technology that enables mutational analysis, gene sequencing, and protein expression testing. They consist of many small arrangements called microarrays that contain DNA, ribonucleic acid (RNA), or protein affixed to a small wafer such as that used in computers. Each microarray, or chip, contains thousands of different sequences of nucleotides or proteins. When a gene chip is reacted with a sample of unknown nature, only complementary sequences of DNA bind to the chip; unbound strands are washed away. One illustration of a gene chip's utility is that by using a gene chip with different tumor-associated genes, it is possible to determine whether a mutant gene, or oncogene, is present in a suspected cancer cell. Biochips are thus useful for identifying potential new drug targets. 4,5

Once a target with a pivotal role in disease is identified, the next step requires designing a drug that will interact with it and deliver a therapeutic effect. Understanding ligand-receptor interaction is a key element in designing a drug to interact with a target. To bind with the target, most drug molecules insert themselves into a functionally critical site of the target protein, like a key in a lock. The molecule then either induces or, more commonly, inhibits the protein's function. Thus, a better understanding of the target's structure and functionality is key to designing better therapeutics, or ligands, that bind to the target. In recent years, better understanding of protein structure and function has yielded sophisticated approaches to the generation and optimization of drug candidates. These methods are commonly referred to as rational drug

design. In essence, rational drug design tailors drug candidates to their target proteins by first elucidating the three-dimensional structure, the binding site, and the active site of the target. 6 Next, medicinal chemists apply combinatorial chemistry techniques and high-throughput screening tools to generate large libraries of compounds whose structure corresponds to the target's strategic site. With the use of biologic assays that reflect the activity of the target protein, researchers can modify the drug candidate to achieve the ideal in vitro effect and test antitargets to determine the drug's specificity.

Biotechnology Drug Classes and Selected Drugs in the Pipeline

A snapshot of some drug candidates in development is beneficial to the understanding of how the technologies discussed in this article are used in drug development and in highlighting areas in medicine in which they may have a significant impact in the near future. We analyzed publicly available information, including the medical literature as well as U.S. Food and Drug Administration (FDA) and drug company reports, to generate a representative but by no means exhaustive list of drug candidates currently in clinical trials. To facilitate this discussion, we found it valuable to assign drugs into classes according to their chemical composition.

Small-Molecule Drugs

SMDs normally have limited biologic interaction capability and less specificity than other drugs for desired targets. In general, an SMD acts as a "spoiler," because its therapeutic effect is limited to the inhibition of an effector protein. For example, by interacting with a hormone receptor, the SMD can inhibit the binding activity of the respective hormone by occupying its docking site or by causing a change in the receptor's three-dimensional configuration. Both patients and the pharmaceutical industry favor the use of SMDs rather than other modalities because of their attractive pharmacokinetic properties, especially their suitability for oral administration and ease of development. 7 SMDs are well positioned to target intracellular proteins (ie, enzymes), because cell membrane penetration is often feasible.

The tyrosine kinase inhibitor STI571 (imatinib mesylate, Gleevec; Novartis Pharmaceuticals Corp., East Hanover, NJ) is an SMD that has had an exceptional impact on the management of Philadelphia-positive chronic myelogenous leukemia (ie, Bcr-Abl-positive) and gastrointestinal stromal tumors with Kit mutations. 8,9 STI571 was first developed to target the platelet-derived growth factor receptor but then was found to be an inhibitor of a specific target protein: the Bcr-Abl protein kinase. Bcr-Abl had previously been implicated in the pathogenesis of Philadelphia mutation-positive leukemia, and STI571 was then developed through a rational process of screening and refining potential small molecules. It therefore serves as a fitting example of the way in which rational drug design is effecting drug development and disease management. 10 Selected SMDs that are currently in clinical trials for different applications are listed in Table 1. 11-30

Protein Drugs

Protein drugs can be subdivided according to their mechanism of action. This categorization scheme helps to create order in the world of protein drugs, yet overlaps exist between different subtypes. Until the early 1970s, proteins were derived from animals or were manufactured synthetically. This process greatly limited their use as pharmaceuticals because of availability and cost issues. Recombinant DNA technology significantly changed these circumstances. Scientists could now insert into a bacterial or yeast cell the human DNA comprising a gene and use the host to manufacture the human protein. With the use of that technology, the bottleneck in protein therapeutics shifted from proteinmanufacturing to the identification of drug targets and the generation of drug candidates.

Therapeutic Hormones and Enzymes

Hormones have been an important subject in pharmaceutical research, because the biology of various hormone deficiencies is relatively straightforward, and thus animal models can be created for research purposes. For instance, insulin was discovered in 1921, leading to the advent of replacement therapy with porcine insulin and a better understanding of diabetes. Furthermore, because hormones are circulating entities, they are more accessible than other proteins-especially intracellular proteins-for research purposes. Hence, early protein therapeutics targeted hormone deficiency states, and when recombinant DNA technology emerged, it was used to manufacture replacement proteins, whose role in disease wasrelatively clear. Enzymes have enormous potential to serve as pharmaceuticals because of their vast number and ubiquitousness. That many enzymes operate intracellularly poses practical problems, however, because protein delivery into the cell is currently next to impossible. More intracellular enzymes are continually being discovered, and methods of enabling oral and intracellular protein delivery are among the most burning challenges in biotechnology. 31 Notable protein drugs that are in the advanced development to launch stages include a T cell-modulating fusion protein for patients with psoriasis and various other autoimmune diseases, as well as recombinant natriuretic peptide for the treatment of patients with congestive heart failure (see Table 2). 33-47

Monoclonal Antibodies

Antibodies (immunoglobulins) are proteins manufactured by B-lymphocytes. They consist of a highly diverse binding site known as the variable region (Fab), which sticks to a corresponding antigen, and a crystallizable fragment domain (Fc), which determines the antibody's functionality. MAb are products of a distinct clone of B cells and are usually derived by immunizing mice against the desired antigen. The reactive B cells are fused with myeloma cells to create hybridomas, which are essentially clones of immortalized B cells that share specificity for the same antigen. 48 The hybridomas' identical product antibody is called a monoclonal antibody, or MAb.

The ability to manufacture clones of identical MAb enables different strategies to create MAb therapeutics. By recruiting the immune system's lytic action, Mab lead to the destruction of antigens implicated in disease. MAb can be used to target toxic drugs such as chemotherapy directly at their site of action, thereby reducing side effects and the required dosage. MAb can be used as diagnostic agents to locate, for instance, residual tumor cells after surgery. 49

In recent years, MAb have made the leap from promising investigational therapies to the clinic, and currently there are 12 approved MAb in the United States. Eight of those 12 MAb are approved for therapeutic indications, including cancer, autoimmune disease, viral infection, and myocardial infarction. In addition, four MAb have been approved for use as diagnostic agents. Most of these have had a significant impact on disease, as evidenced in the discussion of selected MAb in the next few paragraphs.

Rituximab (Rituxan; Genentech, Inc., South San Francisco, CA), the first Mab approved for the treatment of cancer patients, is indicated for non-Hodgkin's B cell lymphoma. It is directed against the cytosine deaminase 20 (CD20) protein, which is found on the surface of normal and malignant B cells. As a single agent, rituximab induces meaningful responses in approximately one-half of patients with relapsed indolent lymphomas and in approximately one-third of patients with relapsed aggressive lymphomas. Because it is a nonchemotherapeuticagent, it also presents a relatively benign side effect profile. Rituximab is currently being tested for other B cell disorders. 50,51

Infliximab (Remicade; Centocor, Inc., Malvern, PA) is a chimeric (human-mouse) MAb that neutralizes tumor necrosis [alpha], a proinflammatory cytokine. It is approved for the treatment of patients with rheumatoid arthritis and Crohn's disease. In clinical trials for rheumatic arthritis, infliximab produced significant improvements in all measures of disease, and treatment with infliximab combined with methotrexate was found to be superior to treatment with methotrexate alone. 52 In Crohn's disease, clinical trials showed infliximab to be effective in producing and maintaining a clinical response in patients with refractory moderate to severe disease. 53

Trastuzumab (Herceptin; Genentech, Inc.) is a humanized MAb that targets the extracellular portion of the human epidermal growth factor receptor 2 (HER2)/Neu receptor. The latter is a member of the EGF receptor family, the blockade of which inhibits the growth of tumors that express it. HER2/Neu is overexpressed in 25 to 30% of breast cancers, increasing tumor aggressiveness. In HER2/Neu-positive patients, the use of trastuzumab with chemotherapy was associated with improved time until disease progression and with overall survival. 54 Several promising MAb currently in late-stage clinical trials are targeting non-Hodgkin's lymphoma, asthma, psoriasis, and different solid organ tumors (Table 2). 33-47

Cytokines

Cytokines are proteins that regulate cells that belong to the immune

system, such as lymphocytes and macrophages. Cytokines have a pivotal role in normal and disease mechanisms in which immune processes play a role, including chronic infectious, autoimmune, cancer, and coronary heart diseases. Many cytokines and cytokine inhibitors are available or are being developed as therapeutics. 55-59

Interleukins (ILs) and interferons are a large and varied family of compounds produced by lymphocytes, macrophages, and monocytes. The FDA has approved the use of recombinant IL-2 for the treatment of patients with renal cell carcinoma, but IL-2 is highly toxic because of its central role in the immune system, which thus far has limited its impact. 60 The FDA has approved the use of recombinant interferons for patients with human immunodeficiency virus-related Kaposi's sarcoma, genital warts, hairy cell leukemia, and hepatitis B and C. 59

Colony-stimulating factors (CSFs) stimulate bone marrow stem cells to differentiate toward a particular cell type. Recombinant versions of CSF, including granulocyte-macrophage CSF, granulocyte CSF, and erythropoietin have revolutionized the ability to treat myelosuppression. Most notably, these agents have had a significant impact in cancer treatment, in which myelosuppression is a common complication of chemotherapy. Proved and suggested effects of treatment include shortening the duration of febrile neutropenia after myelosuppressive treatment, mobilization of hematopoietic stem cells for ensuing transplantation, and reductions in chemotherapy-associated infections, antibiotic use, hospital stay, and mortality. 61,62 Erythropoietin has had a profound effect on the treatment of patients with end stage renal failure-associated anemia. 63 Some investigational cytokines are listed in Table 2.

Gene Therapy

Gene therapy may be defined as the transfer of recombinant DNA into human cells to achieve the production of a desired protein. Depending on the strategy used, DNA may be introduced into cells removed from the body (ie, the ex vivo approach) or directly into cells in their normal location (ie, the in vivo approach). 64 Gene therapy has various potential applications, such as treating patients with enzyme deficiencies or cancer. Efficient gene transfer requires the use of a vector. All vectors contain, at a minimum, the transgene of interest linked to a promoter to drive its expression. 65 Increasingly wider ranges of viral and synthetic vectors are available, each of which has characteristic advantages and limitations. Generally, viral vectors achieve better transfection than other vectors but have other problems such as immunogenicity and complicated manufacturing. Liposomes are nonviral vectors that mitigate the immunogenicity problem but provide less efficient transfection and protein expression. Naked DNA is a third method that uses plasmids, which usually are administered by direct injection into tumor or muscle as opposed to systemic delivery. 66

There are several strategies whereby gene therapy may be used to treat cancer. In the corrective gene therapy approach, when malignant transformation is associated with inactivity of tumor suppressor genes such as p53 and p21, supplying tumors with the intact gene may reverse malignant transformation by promoting apoptosis. 67-69 Another strategy is cytoreductive gene

therapy, in which immunotherapy or cytolytic/proapoptotic approaches are used. Immunotherapy uses gene transfer to facilitate a dormant host immune response directed against the tumor. Evasion of autologous host cellular immunity is a common feature of tumor cell neoantigens, because tumor cells are poor antigen-presenting cells. Cancer vaccine strategies are based on optimization of the context in which tumor antigens or tissue-specific antigens are presented to the host immune system. When appropriately primed, the activated host immune system can then act against tumor cells systemically.

The ex vivo approach starts with inactivated cancer cells obtained from the patient. Different techniques are then used to enhance the immunogenicity of tumor-specific antigens, including growth in a cytokine-rich environment, coinjection of tumor cells along with cytokines back into the patient, or transfection of these cells in vitro with genes that encode immunostimulatory cytokines. A second approach is to administer an injection of a purified tumor-associated protein or peptide into the patient, without injecting the entire tumor cell. The third approach uses in vitro manipulation of host antigen-presenting cells such as dendritic cells, which are involved in initiating the T cell-mediated response against antigens. Confronting dendritic cells with the desired antigen in vitro stimulates an immune response upon injection into the patient. 70,71 Enzyme/prodrug gene therapy, also referred toas suicide gene therapy, relies on the conversion of an inactive prodrug into a toxic drug with the use of an enzyme vectored only to the target tumor cells. In this way, active drug is limited spatially to the transduced cells and adjacent surrounding cells, facilitating higher tumor drug concentrations without increased normal tissue toxicity. Prodrug-activating enzymes that have been used in this approach include cytosine deaminase, which catalyzes the conversion of the nontoxic 5-fluorocytosine to the cytotoxic 5-fluorouracil, and herpes simplex virus thymidine kinase, which, together with cellular enzymes, facilitates the conversion of ganciclovir into the toxic ganciclovir triphosphate.

Viral vectors may themselves be designed to target and kill tumor cells without the insertion of a foreign transgene (eg, oncolytic viruses). The adenovirus life cycle includes a lytic phase, which can result in host cell death independent of entry into the cell cycle. Adenovirus has evolved a potent repertoire of gene products that may exert profound effects on the growth regulation of the host cell to facilitate viral replication. The ONYX-015 vector, a replication-competent adenovirus designed to preferentially replicate in p53 mutant cells, is currently in clinical trials (Table 3). 72 ONYX-015 is the first genetically engineered replication-competent virus to demonstrate selective intratumoral replication and necrosis in patients. 73

Antisense Drugs

Although traditional drugs are designed to interact with protein molecules, antisense drugs are designed to inhibit the production of disease-causing proteins. During the transcription of information from DNA to messenger

RNA (mRNA), two complementary strands of DNA partly uncoil such that one strand is used as a template for the transcribing enzymes, which assemble mRNA in a process called transcription. mRNA then migrates into the cell, where its encoded information is read by the ribosomes and translated to the specific protein. 95

Antisense drugs are complementary strands of small segments of mRNA. To create antisense drugs, nucleotides are linked in short chains (ie, oligonucleotides). Each antisense drug is designed to bind to a specific sequence of nucleotides in its mRNA target to inhibit the production of the protein encoded by the target mRNA. Fomivirsen (Vitravene; Isis Pharmaceuticals, Inc., Carlsbad, CA) is an antisense strand complementary to the mRNA of a crucial cytomegalovirus protein. It is an FDA-approved medication indicated for patients with acquired immunodeficiency syndrome-related cytomegalovirus retinitis. Oblimersen sodium (Genasense; Genta, Inc., Berkeley Heights, NJ) is an antisense drug that binds to the Bcl-2 mRNA, which is expressed by different cancers (Table 3).

Conclusions

The development of a new drug requires the identification of a protein target, techniques for the generation of compounds that react with the target in a desired fashion, and innovative delivery mechanisms by which to lead the drug to its target. The tools of biotechnology are effecting advancements on all of these fronts. An abundance of new gene and protein targets that can be targeted by therapeutics are being investigated. Moreover, whereas in the past most drugs were randomly generated small-molecule compounds that were limited to the blockade of certain pathways, other drug classes have emerged, including recombinant protein drugs and MAb, DNA and cellular vaccines, gene therapy, and antisense therapy. SMDs, which remain a fundamental weapon against many diseases, can be engineered to provide a better therapeutic profile than before. Some of the newer drug classes, including protein drugs and MAb, already have exhibited proof of concept as approved drugs on the basis of several years of experience. The potential impact on disease of cancer vaccines, gene therapy, and antisense therapy remains to be determined, but there seems to be consensus regarding the eventual important role of these technologies.

As a result of these developments, physicians will be able to attack the same target with a mix of various drug classes, such as combinations of MAb or a cancer vaccine against a tumor-associated protein, a cytokine to increase the antitumor immune response, and gene therapy encoding for a suicide protein. Such an approach is not yet feasible, but the drugs that will allow experimentation with such combinations are at our doorstep.

Several drug candidates are far along the route to becoming FDA-approved drugs. These include a cellular vaccine for hormone-resistant prostate cancer, a cancer vaccine and a small-molecule antiangiogenic drug for renal cell carcinoma, a new immune modulator protein drug directed against psoriasis and other autoimmune diseases, an SMD directed against transitional cell carcinoma, and several Mab that target cancer, autoimmune disease, and graft versus host disease. Judging from the wealth of advanced clinical candidates in the pipeline, the impact of biotechnology on the practice of medicine will soon increase markedly,

empowering clinicians with new ways to fight disease.

Acknowledgment

NJM is a recipient of a fellowship from The American Physicians Fellowship for Medicine in Israel.

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Better to be kind at home Than to burn incense in a distant place. --Chinese proverb

Key Words: biotechnology; drug classes; drug development

Accession Number: 00007611-200312000-00005

Version: 7.1.375 / Virus Database: 267.15.2/251 - Release Date: 2/4/2006

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