Journal of Cellular Biochemistry 102:580–592 (2007)

Targeting Cell Signaling Pathways for Drug Discovery: An Old Lock Needs a New Key

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Abstract In this age of targeted therapy, the failure of most current drug-discovery efforts to yield safe, effective, and inexpensive drugs has generated widespread concern. Successful drug development has been stymied by a general focus on target selection rather than clinical safety and efficacy. The very process of validating the targets themselves is inefficient and in many cases leads to drugs having poor efficacy and undesirable side effects. Indeed, some rationally designed drugs (e.g., inhibitors of receptor tyrosine kinases, tumor necrosis factor (TNF), cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF), bcr-abl, and proteasomes) are ineffective against cancers and other inflammatory conditions and produce serious side effects. Since any given cancer carries mutations in an estimated 300 genes, this raises an important question about how effective these targeted therapies can ever be against cancer. Thus, it has become necessary to rethink drug development strategies. This review analyzes the shortcomings of rationally designed target-specific drugs against cancer cell signaling pathways and evaluates the available options for future drug development. J. Cell. Biochem. 102: 580–592, 2007.

Key words: cancer; targeted therapy; cell signaling; drug-discovery; natural products

Abbreviations used: AP-1, activator protein-1; CDK, cyclin-dependent kinase; COX-2, cyclooxygenase-2; EGF, epidermal growth factor; ICAM-1, intercellular adhesion molecule-1; JNK, c Jun N-terminal kinase; MMP, matrix metalloproteinase; MAPK, mitogen-activated protein kinases; NF-κB, nuclear factor-kappa B; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; PARPPoly (ADP-ribose) polymerase; PPAR-γ, peroxisome proliferator-activated receptor gamma; XIAP, X-linked inhibitor of apoptosis; HDAC, histone deacetylase; Egr-1, Early growth response; RANKL, receptor activator of NFkappaB ligand; IKK, IκBα kinase; PKC, protein kinase C; Nrf2, NF-E2-related factor 2; GST, glutathione S-transferase; JAK2, janus kinase; ELAM1, endothelial leukocyte adhesion molecule 1; VCAM-1, vascular cell adhesion molecule-1; MDR, multidrug resistance; uPA Urokinase-type plasminogen activator; IL, interleukin; LOX, lipoxygenase; PK, protein kinase; iNOS, inducible nitric oxide synthase; NAG, nonsteroidal anti-inflammatory drug-activated gene; ATF, activating transcription factor.

Grant sponsor: Clayton Foundation for Research; Grant sponsor: Department of Defense US Army Breast Cancer Research Program; Grant number: BC010610; Grant sponsor: NIH P01; Grant number: CA-91844; Grant sponsor: NIH P50 Head and Neck Cancer SPORE; Grant sponsor: NCI Cancer Center Core; Grant number: CA-16672.

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Received 21 June 2007; Accepted 25 June 2007
DOI 10.1002/jcb.21500

Since l971, when President Richard Nixon signed the National Cancer Act into law and made the "conquest of cancer a national crusade," over $200 billion have been spent, over 1.5 million research studies published, and over 150,000 animal studies performed to find a cure for cancer. Yet, while the incidence of cardiovascular, infectious, and cerebrovascular diseases has decreased significantly, that of cancer has remained unchanged [Howe and Clapp, 2004]. Why is this so? Is cancer a more complex and challenging disease? Have cancer research efforts been misguided? In any case, what is the future of cancer research?

Diseases are characterized by dysregulation of biological pathways [Baylin and Ohm, 2006] that can result from infections, environmental factors, genetic mutations, or lifestyle. Such dysregulation alters the expression of proteins in multiple cellular pathways, leading to changes in growth, differentiation, or apoptosis. Thus, it has always been a challenging task to identify drugs that can restore affected individuals to a healthy state. Traditionally, the approach to drug discovery has been physiologically based and usually aimed at systemic targets. However, when in 1908 Paul Ehrlich launched his pioneering search for "magic bullets" that could selectively target the constituents of infectious organisms relative to the host's [Ehrlich, 1913], there began a gradual shift from the use of complex extracts to the use of defined small molecules [Albert, 1968] and toward today's targeted therapies. The present article will review the current standing and efficacy of existing targeted therapeutics, their shortcomings, and the future of drug discovery.

TARGET-SPECIFIC DRUGS

The main goal of using a target-specific drug is to inhibit a molecular target central to a disease mechanism of interest. The first step toward this goal is to identify individual molecular targets and validate their relevance to the human disease. This is followed in turn by identification of specific chemical- or antibody-based small molecule modulators or inhibitors of the target. Target validation is a complex and extremely difficult process [Tobert, 2003], and extremely few disease-relevant targets are amenable to drug treatment [Hopkins and Groom, 2002]. A target is usually a single gene, gene product, or signaling pathway that has been identified on the basis of genetic analysis or biological observations [Kerns and Di, 2003; Knowles and Gromo, 2003; Lindsay, 2003]. In theory, targeting a single molecular mechanism should be sufficient to achieve a significant therapeutic effect; in reality, however, single-target drugs have had very little therapeutic impact. In fact, they have generally been highly ineffective in treating complex diseases (e.g., cancer) [Hanahan and Papahadjopoulos, 1965] or diseases affecting multiple tissues or cell types (e.g., diabetes and immunoinflammatory disorders).

The search for cancer drugs has traditionally focused on the molecular signaling pathways that go berserk in cancer cells. These signaling pathways rely heavily on the action of over 500 protein kinases whose dysregulation has been implicated in cancer [Manning et al., 2002; Krause and Van Etten, 2005]. Thus, developing drugs that target signaling pathways has become an attractive venture for pharmaceutical companies and biotechnology industries. For example, the receptor tyrosine kinases epidermal growth factor receptor (EGFR) and ErbB2 (Her-2) receptor were cloned in 1983 by researchers at Genentech, Inc., and monoclonal antibodies were subsequently generated to target them. These monoclonal antibodies were then developed into the drugs cetuximab (Erbitux, C-225, BMS; Merck) and trastuzumab (Herceptin; Genentech), respectively. These drugs were approved by the FDA in 2004 for the treatment of colon cancer. The Bcr/Abl kinase inhibitor imatinib mesylate (Gleevec; Novartis) was approved in 2001 for the treatment of chronic myelogenous leukemia (CML). This was followed by development of several other target-specific drugs (Table I). Except for Gleevec, the other single-target drugs developed so far have demonstrated poor safety and efficacy profiles and turned out to be prohibitively costly.

EGFR Inhibitors

EGFR was the first tyrosine kinase receptor to be linked directly to human tumors [Gschwind et al., 2004]. In many tumors EGF-related growth factors are produced by the tumor cells themselves or are made available by surrounding stromal cells, leading to constitutive EGFR activation [Salomon et al., 1995]. Gene amplification leading to EGFR overexpression often

TABLE I. FDA-Approved Cancer Drugs And Their Molecular Targets, Therapeutic Uses, Side Effects, And Cost

Target	Drug	Use	Side-effects	Cost[a] ($)/month	Market sale[b] (millions)
CD52	Campath (alemtuzumab)	CLL	Blood disorder	66,456	—
VEGF	Avastin (bevacizumab)	Colorectal cancer	Neurological disorder	32,976	1,700
CD20	Rituxan (rituximab)	NHL	Skin disease	15,904	2,100
IL-2 receptor	Zenapax (daclizumab)	Leukemia	Allergy	11,832	—
p185 neu	Herceptin (trastuzumab)	Breast cancer	Heart attack	5,858	1,200
Proteasome	Velcade (bortezomib)	Multiple myeloma	GI disorder	5,160	220
TNF	Remicade	Crohn's disease/Arthritis	Lymphoma risk	3,500	3,000
TNF	Humira	Arthritis	Allergy	2,880	2,000
TNF	Enbrel	Arthritis	Allergy	1,440	2,900
Bcr/Abl	Gleevec (imatinib mesylate)	CML	Heart and bone	3,204	1,200
EGFR antibody	Erbitux (cetuximab)	Colorectal cancer	Allergy	11,520	550
EGFR kinase	Tarceva (erlotinib)	NSCLC	Rash and Diarrhea	3,249	650
EGFR kinase	Iressa (gefitinib)	NSCLC	Interstitial lung disease	2061	400
COX-2	Celebrex (celcoxib)	Colon cancer	Heart attack	250	2,000

CLL, chronic lymphocytic leukemia; VEGF, vascular endothelial growth factor; NHL, Non-Hodgkin lymphoma; CML, chronic myelogenous leukemia; EGFR, epidermal growth factor receptor; IL-2 R, interleukin-2 receptor; GI, gastrointestinal; EGFRTK, epidermal growth factor receptor tyrosine kinase; NSCLC, non-small-cell lung carcinoma, TNF, tumor necrosis factor; RAr, rheumatoid arthritis; COX, cyclooxygenase.
[a]Based on average wholesale price from red book.
[b]Market sales reported in 2006.

occurs in human cancers. Furthermore, mutations in the EGFR have been observed in gliomas [Ekstrand et al., 1992] and carcinomas of the breast, lung, and ovaries [Moscatello et al., 1995]. Three types of EGFR inhibitor are currently approved by the FDA for the treatment of cancers. Geftinib (Iressa, ZD1839; Astra Zeneca), and erlotinib (Tarceva, 051-774; Genentech) are ATP competitive inhibitors of the tyrosine kinase domain of EGFR [Fukuoka et al., 2003; Shepherd et al., 2005]. EGFR is also the target of cetuximab, a chimeric humanized monoclonal antibody specific for EGFR's extracellular domain. Cetuximab is already approved for use against colorectal cancers refractory to irinotecan [Cunningham et al., 2004].

Iressa won FDA approval in 2003 on the basis of several dramatic responses in phase II lung cancer trials, but its combination with chemotherapy in phase III trials has conferred no survival benefit. Somatic mutations in the tyrosine kinase domain of EGFR were recently identified in non-small-cell lung cancers in a subgroup of patients who responded clinically to treatment with gefitinib and erlotinib. Compared with placebo-treated patients, patients treated with erlotinib experienced significantly better response rates (8.9% vs. <1%) and better response durations (median, 7.9 months vs. 3.7 months), progression-free survival (2.2 months vs. 1.8 months), and overall survival (6.7 months vs. 4.7 months) [Shepherd et al., 2005]. Yet, despite its apparent efficacy, the "wonder drug" erlotinib had to be stopped in 5% of patients because of toxic side effects and in the end provided a survival advantage over placebo of only 2 months. Thus, while they have added to our experience, erlotinib and other single-target drugs have hardly turned out to be cures. Moreover, they have often turned out to be very expensive and sometimes unaffordable: for example, a 4-month course of cetuximab costs approximately $38,000.

Amplification of ErbB2, another member of EGFR superfamily, occurs in 25–30% of breast cancer patients. Trastuzumab (Herceptin; Genentech), a humanized monoclonal antibody that acts on the HER2/neu (erbB2) receptor, was the first protein kinase inhibitor to be approved for the treatment of cancer [Piccart-Gebhart et al., 2005]. However, as for all other kinase inhibitors, herceptin works well only in a small percentage of patients with

breast cancer, and those who do respond generally acquire resistance within 1 year. To improve the efficacy of trastuzumab in breast cancer patients, it will be critical to elucidate the mechanism of resistance in these tumors and to develop strategies in which it is combined with chemotherapeutic agents or other novel modalities.

Bcr-Abl Inhibitors

Gleevec is a small molecule inhibitor that binds the inactive form of Bcr-Abl. It was approved in May 2001 for the treatment of CML, a cancer associated with a de novo translocation mutation (i.e., the Philadelphia chromosome) that results in production of a functional Bcr-Abl kinase. Developed as a specific inhibitor of this disease-causing mutant kinase, Gleevec made a good initial impact until tumor cells became resistant to it [Druker et al., 2006].

Vascular Endothelial Growth Factor (VEGF) Inhibitors

Bevacizumab (Avastin; Genentech) is a recombinant humanized monoclonal antibody that binds to and inhibits the biologic activity of human vascular endothelial growth factor (VEGF), a factor that stimulates the formation of new blood vessels. It was approved in 2004 as first-line therapy for patients with metastatic colorectal cancer [Hurwitz et al., 2004]. A 10-month course was shown to extend patients' lives by only 5 months when given intravenously in combination with standard chemotherapy drugs for colon cancer (Table II). In a phase II trial in patients with advanced pancreatic cancer, bevacizumab in combination with gemcitabine resulted in only 6 months' survival and the pretreatment plasma VEGF levels did not correlate with outcome [Kindler et al., 2005]. In another randomized study on patients with recurrent or advanced non-small-cell lung cancer, the patients achieved only 2 month survival advantage with bevacizumab and the VEGF levels before treatment did not correlate with overall survival [Sandler et al., 2006]. Unfortunately, this meager survival advantage was achieved at exorbitant cost (approximately $49,000 per 10-month course) and was associated with several serious and occasionally fatal complications (e.g., gastrointestinal perforation and wound dehiscence in patients with colorectal cancer and hemoptysis in non-small-cell lung cancer patients).

TABLE II. Survival Advantage, Response Rate, and Toxicity of Recent FDA-Approved Target-Specific Drugs

Drug	Cancer	Survival advantage (months)	Response rate in patients (%)	Toxicity	Reference(s)
EGFR inhibitor					
Tarceva	Pancreatic	0.3	8.6	Hemoptysis	[Moore et al., 2007]
Tarceva	NSCLC	0.4	7.0		[Shepherd et al., 2005]
Iressa	NSCLC	2.7	8.0	Fatal interstitial lung disease	[Fukuoka et al., 2003; Inoue et al., 2006]
Erbitux	Colorectal	1.7	10.8	Acne-like rash, folliculitis	[Cunningham et al., 2004],
VEGF inhibitor					
Avastin	Pancreatic	0.6	NA	Hypertension, thrombosis, proteinuria	[Burtness, 2007; Kindler et al., 2005; Kindler et al., 2007]
Avastin	Colorectal	2.0	2.1		[Giantonio et al., 2007]
Avastin	NSCLC	2.0	35		[Sandler et al., 2006]
HER2 inhibitor					
Herceptin	Breast	5.1	15	Heart failure	[Cobleigh et al., 1999; Eiermann, 2001; Romond et al., 2005]
Proteasome inhibitor					
Velcade	Multiple Myeloma	16	35	Thrombocytopenia, peripheral neuropathy, neutropenia	[Richardson et al., 2003]
Bcr-abl inhibitor					
Gleevec	CML	21	60	Hypophosphatatemia	[Berman et al., 2006; Druker et al., 2006; Warren et al., 2004]

EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor; NSCLC, non-small-cell lung carcinoma; NA, not available.

Tumor Necrosis Factor (TNF) Inhibitors

Several tumor necrosis factor (TNF) inhibitors have been approved by the FDA. Infliximab (Remicade; Centocor, a subsidiary of Johnson & Johnson) is a chimeric monoclonal antibody that reduces the amount of active TNF-α in the body by binding it and preventing it from signaling cell-surface receptors for TNF. Etanercept (Enbrel; Amgen) is a recombinant human soluble TNF-α receptor fusion protein. Etanercept was approved by FDA in 2002 for the treatment of inflammatory disorders involving overexpression of TNF (e.g., autoimmune diseases, psoriatic arthritis, rheumatoid arthritis). Another FDA-approved TNF antagonist is adalimumab (Humira; Abbott), which unlike infliximab (mouse-human chimeric antibody) and etanercept (TNF receptor-IgG fusion protein) is constructed from a fully human monoclonal antibody. Adalimumab has been approved for the treatment of Crohn's disease and other autoimmune disorders. Both etanercept and infliximab have been used at M. D. Anderson for the non-FDA-approved indication of graft-versus-host disease management.

Unfortunately, these drugs are expensive too. Etanercept injected subcutaneously in two doses of 25 mcg each costs approximately $1,600. Infliximab administered to a 65-kg patient at a dose of 10 mg/kg IV weekly for up to eight doses costs approximately $9,000. Moreover, their use has been associated with severe side effects that include fatal blood disorders, infections, and diseases and rare instances of lymphoma, solid tissue cancer, serious liver injury, and demyelinating central nervous system disorders. Consequently, the FDA has issued a warning regarding the product labeling of these drugs.

Thalidomide, another inhibitor of TNF, has also been approved by FDA for the treatment of newly diagnosed multiple myeloma. However, thalidomide's clinical effectiveness is questionable. In a trial comparing high-dose therapy for myeloma patients with and without thalidomide added, thalidomide extended 5-year event-free survival by only 8% and did not improve overall survival; more ominously, it increased the incidence of severe peripheral neuropathy and deep-vein thrombosis [Barlogie et al., 2006].

Proteasome Inhibitors

Proteasome inhibitors target cellular enzymes known as proteasomes that help regulate cell function and growth. The proteasome inhibitor bortezomib (Velcade; Millenium Pharmaceuticals), which modulates cell signaling by inhibiting the proteasomes, has been approved for the treatment of multiple myeloma, but only in individuals who have already received two other types of chemotherapy. Whereas the number and type of prior therapies do not appear to influence treatment response rates, other factors (i.e., plasma cell levels >50% or abnormal bone marrow cytogenetics) do appear to negatively affect them. Interestingly, however, responses have been seen in patients with chromosome 13 abnormalities. Bortezomib is efficacious, as demonstrated by a median survival of 16 months in recipients, and reasonably successful in treating multiple myeloma up to a certain level. However, because it also regulates the function of 400 genes whose expression is both beneficial and detrimental to patients, it may cause side effects such as peripheral neuropathy, orthostatic/postural hypotension, gastrointestinal adverse events, thrombocytopenia, and cardiovascular toxicity [Richardson et al., 2003].

Cyclooxygenase-2 (COX-2) Inhibitors

Cyclooxygenase enzymes are responsible for catalyzing the conversion of arachidonic acid into prostaglandins, which in turn have been implicated in inflammation and cancer. Cyclooxygenase-2 (COX-2) levels are often found to be elevated in inflammatory diseases. Celecoxib (Celebrex; Pfizer) was developed as a specific inhibitor of COX-2 and marketed as a COX-2-targeted drug. It was approved by the FDA in 1998 for relief of the signs and symptoms of osteoarthritis and adult rheumatoid arthritis and was subsequently used extensively to manage osteoarthritis, adult rheumatoid arthritis, acute pain, and painful menstrual cycles. It was also used to reduce the number of colorectal polyps in patients with familial adenomatous polyposis (FAP), an inherited disease in which the rectum and colon are covered with polyps. Indeed, celecoxib treatment led to remarkable reductions in mean polyp number (28%) and size (31%) [Steinbach et al., 2000]. (Interestingly, celecoxib's clinical activity in FAP patients was recently exceeded by that of curcumin (a naturally occurring substance derived from curry spice), which reduced polyp size and number by 60% [Cruz-Correa et al., 2006].) Despite its design and

nominal status as a COX-specific inhibitor, celecoxib can in fact bind both COX-2 and COX-1 (although it is approximately eight times more selective for COX-2 than for COX-1) and can bind both COX-2-positive and -negative cells, thus, raising serious questions about its actual specificity. In addition, celecoxib has turned out to be extremely toxic to the heart, liver, kidneys, and stomach, which led to its black box labeling by the FDA in 2005. A general lack of efficacy and serious side effects have limited the use of this drug.

HMG-CoA Reductase Inhibitors

Cholesterol biosynthesis in mammals is a complex process involving more than 30 enzymes. Although not thoroughly investigated, increased cholesterol concentration in the blood is understood to be a risk factor for coronary heart disease, and it is natural that the search for drugs to reduce plasma cholesterol concentrations has focused on steps in the cholesterol synthesis pathway. Being the rate-limiting enzyme in cholesterol biosynthesis, HMG-CoA reductase was logically targeted to develop drugs that could modulate cholesterol biosynthesis. The first potent HMG-CoA reductase inhibitor was isolated from the fungus *Aspergillus terreus* in 1978 by researchers at Merck Research Laboratories and given the name Lovastatin [Alberts et al., 1980]. By 1987, this new class of inhibitors, now called statins, were approved for clinical use in treating hypercholesterolemia.

Over the next decade, statins gained a well-earned reputation for safety that was based on hundreds of clinical trials and prescription use by many millions of patients. However, in August 2001, the makers of cerivastatin, which had been introduced in 1998, withdrew it from the market in response to numerous reports of rhabdomyolysis, including more than 50 fatal cases [Ballantyne et al., 2003]. It is now known that high-dose statins are extremely toxic to a variety of animal species and that their toxic effects can include rhabdomyolysis, hepatic transaminase elevation, atypical focal hyperplasia of the liver, cataracts, vascular lesions in the central nervous system, skeletal muscle toxicity, testicular degeneration, and tumors of the liver and other sites.

CD20 Inhibitors

Rituximab (Rituxan; Genentech) is a chimeric anti-CD20 IgG1 monoclonal antibody approved for the treatment of relapsed or refractory diffuse large B-cell lymphomas. When administered alone, rituximab offered an overall survival benefit of only 6 months [Davis et al., 1999]. In combination with the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone), it improved response rates by only 13% [Coiffier et al., 2002].

Inhibitors of Hypertension

Minoxidil was originally developed as an oral vasodilator for the treatment of hypertension [Campese, 1981]. However, its prominent side effect of excessive hair growth consequently led to its ancillary development as a treatment for reversing baldness. The mechanism of action by which minoxidil inhibits hypertension is still unknown. Minoxidil is believed to be a potassium channel agonist that contains within its makeup the chemical structure of the vasodilator nitric oxide (NO), which may explain this drug's ability to stimulate hair growth and reverse baldness. In addition to hair growth, minoxidil's other side effects include very low blood pressure, irregular or fast heart beat, blurred vision, chest pain, and possible transmission from mother to child via breast milk [Valdivieso et al., 1985].

Another targeted inhibitor of hypertension, sildenafil citrate (Viagra), was originally developed by Pfizer for the treatment of pulmonary arterial hypertension and angina pectoris. Although the drug failed to cure angina pectoris, it did eventually find its way to market as an enormously popular and sometimes abused treatment for erectile dysfunction. Sildenafil citrate can cause sudden dangerous drops in blood pressure when taken with nitrates. It can also severely impair liver and kidney function and induce hypotension, stroke, heart attack, and hereditary degenerative retinal disorders.

Other Potential Inhibitors

Several other specific targets for inhibition and rational drug design (e.g., nuclear factor-κB [NF-κB], STAT, and AKT) are currently being explored by pharmaceutical companies (Fig. 1). However, applying the single drug-single target theory to these molecules may not result in effective anticancer drugs.

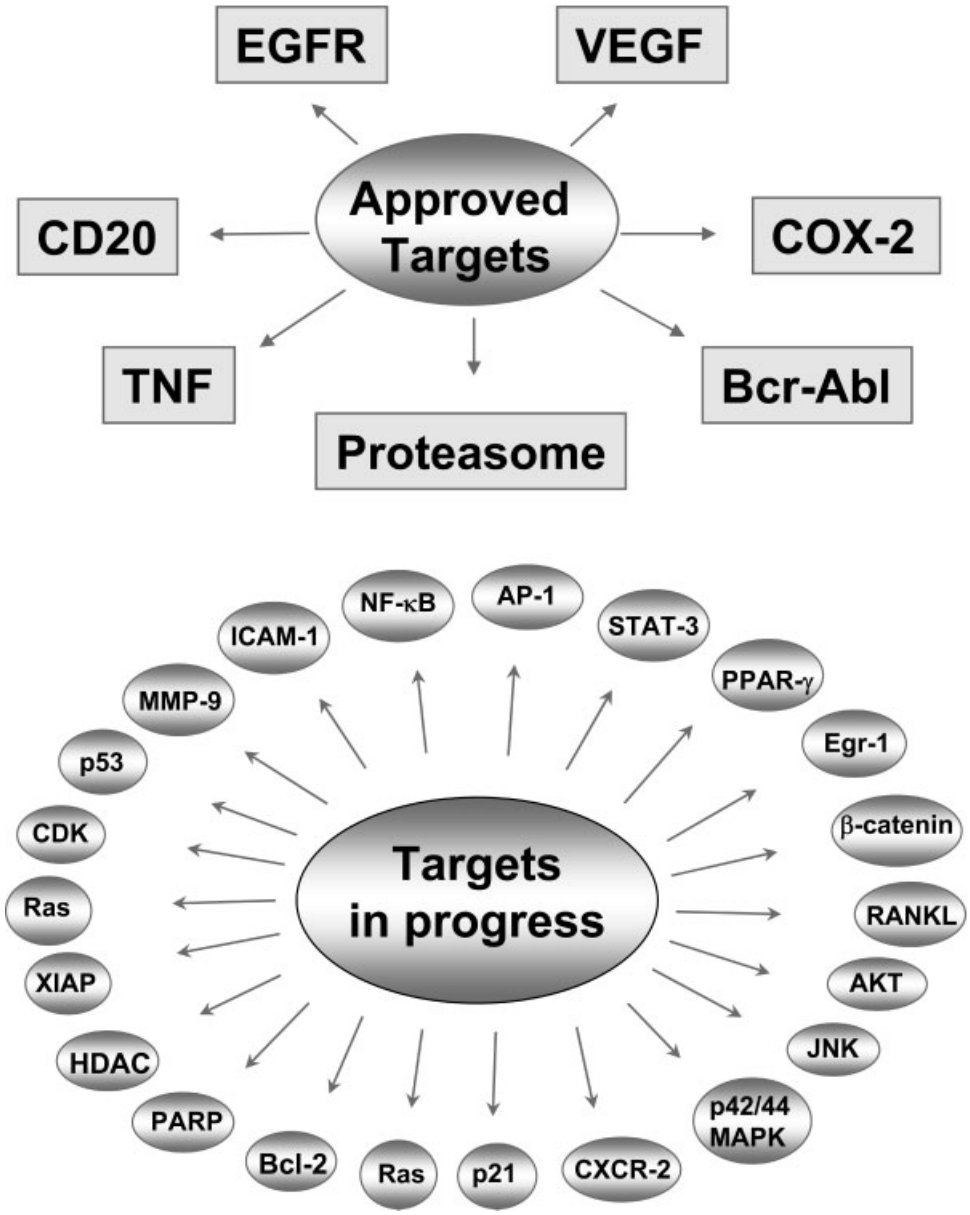

Fig. 1. Targets approved and under investigation for rational drug design.

TARGET-SPECIFIC DRUGS: WHY DO THEY FAIL?

The development of "smart" drugs that target specific signaling pathways has been hampered by poor efficacy, undesirable side effects, and tumor resistance. The most relevant question is why these drugs fail despite years of extensive and time-consuming preclinical and clinical testing. Although the most prevalent human diseases (i.e., cancer, diabetes, heart disease, arthritis, asthma, and depression) are multifactorial in origin and have both genetic and environmental risk factors [Kaplan and Junien, 2000; Reich and Lander, 2001], most modern searches for new drugs to treat them employ the one-target, one-drug paradigm. In brief, research efforts are focused on identifying one single new chemical entity that inhibits one single well-defined molecular target. Interestingly, most of the single-target drugs that have been developed in this way have shown very little efficacy when administered alone but have improved overall survival (though rarely to any significant extent) when given in combination with existing standards of care (Table II)

[Coiffier et al., 2002; Fukuoka et al., 2003; Richardson et al., 2003; Cunningham et al., 2004; Romond et al., 2005; Shepherd et al., 2005; Druker et al., 2006; Giantonio et al., 2007; Moore et al., 2007].

Proponents of the one-drug, one-target approach accept that many drug candidates fail because of incorrect target selection. The Human Genome Project has identified 20,000–25,000 genes, thereby creating an enormous repertoire of potential targets. Several groups, using bioinformatic methods, have hypothesized that 5,000–6,000 of them may be feasible drug targets (for references, see [Hopkins and Groom, 2002]). The challenge is to match gene with disease and validate the target for drug development. At present, the pharmaceutical industry validates targets by utilizing human genetic associations, mouse models, and RNA profiling/RNAi technologies. While this allows specific targeting, it does not always ensure a candidate drug's ultimate safety and efficacy. In fact, a candidate drug's toxicity can only be recognized at the clinical trial stage, usually after enormous resources have been invested in the drug development.

The basic flaw in the one-target, one-drug approach is that it focuses mainly on increased systematic drug screening capacity rather than on consideration of traditional physiology-based concerns. For example, when it was discovered that a mutation in a leptin receptor gene led to obesity, a leptin analogue was developed; unfortunately, the analogue was effective only in people carrying the specific mutation and did not induce weight loss in people lacking it [Yanovski and Yanovski, 2002]. Another example is depression. That most individuals can be treated by various means (e.g., inhibition of serotonin and noradrenalin, inhibition of monoamine oxidase-B, administration of tricyclics, and electroconvulsive therapy) suggests that the treatment does not have to affect a single target, but rather that a similar clinical benefit can be achieved through different mechanisms [Brunello et al., 2002].

That tumorigenesis involves multiple genetic changes is well established. However, two important questions remain unanswered: how many mutations are required for the pathogenesis of a specific tumor [Renan, 1993], and how much of the genome is amenable to targeted drug treatment [Hopkins and Groom, 2002]? It is still not clear whether targeting intracellular molecular signaling pathways would be more advantageous than targeting individual genes and proteins [Fishman and Porter, 2005]. It is believed that intracellular molecular signaling pathways are triggered by extracellular molecules that bind to receptors in the cell membrane, thereby switching on intracellular systems that relay activation, or inactivation, signals to particular genes and ultimately affect a cell's ability to grow and differentiate [Fishman and Porter, 2005]. Yet, cancer drugs designed to target signaling pathways have been hampered by a lack of efficacy and by tumor resistance. For example, the clinical ineffectiveness of anti-TNF antibodies specifically designed to suppress the TNF signaling pathway has raised doubts about whether targeting a single pathway will produce a favorable outcome. Indeed, most signaling pathways engage in extensive cross-talk and intricate interactions with other pathways, which suggests that targeting a single step in any signaling pathway may be futile.

THE FUTURE OF DRUG DISCOVERY

The dismal performance of targeted therapies raises several questions about the future of drug discovery. Can a single drug or target cure a multi-factorial disease like cancer? Can the limitations of single-agent therapies be overcome by attacking a disease on multiple fronts? Drugs aimed at multiple targets can be more efficacious and less vulnerable to acquired resistance because the disease system is less able to compensate for the action of 2 or more drugs simultaneously. Systematically screening combinations of active pharmaceutical ingredients for potential synergy may be especially valuable in this regard [Borisy et al., 2003]. Indeed, drug combinations that reach multiple targets simultaneously are better at controlling complex disease systems, less prone to drug resistance, and the standard of care in many important therapeutic areas [Keith et al., 2005]. Thus, the key to improving future drug discovery may require a trip to the past and a return to historically established means of identifying effective drugs for the treatment of complex diseases.

Multi-Component Therapeutics

Multi-component therapies that combine more than one active ingredient in a clinical

setting have been successfully used in treating a number of diseases including cancer and infectious diseases [Keith et al., 2005]. The individual components create a combination effect by affecting separate targets simultaneously. The targets can occupy the same or separate pathways within an individual cell, or even in separate tissues. One component alters the ability of another to reach its target. The components bind separate sites on the same target to create a combination effect and increase the pharmacological action. This multi-target approach can be particularly beneficial in cancers because oncogenesis is known to be a multi-genic process and because most cancers are known to exhibit at least 4–7 independent mutations [Renan, 1993]. For example, the ErbB2 (HER-2/neu) inhibitor trastuzumab (Herceptin) is now being combined with the anti-VEGF inhibitor bevacizumab (Avastin) to treat breast cancer and the ErbB1 inhibitor cetuximab (Erbitux) with irinotecan to treat colorectal cancer [Hynes and Lane, 2005]. In both cases, the goal is to block several cell growth pathways and overwhelm tumors before they become resistant. The major limitation of these multi-targeted combination therapies, however, is their increased toxicity.

Structural Biology

By revealing new ways for drugs to bind to kinase targets, structural biology is yielding a new generation of inhibitors and pinpointing the sources of drug resistance [Daub et al., 2004]. ATP-competitive kinase inhibitors such as gefitinib (Iressa), erlotinib (Tarceva), and lapatinib are small enough to be made orally bioavailable while still retaining good pharmaceutical properties; however, despite being fairly specific for their targets, they can still bind to other kinases and thereby cause unwanted side effects. Moreover, tumors treated with these kinases eventually acquire mutations in the ATP-binding pocket that interfere with drug binding and ultimately lead to drug resistance and treatment failure. Several pharmaceutical companies are actively exploring novel approaches, including designing compounds to overcome drug resistance originating in the ATP-binding pocket. Still, the question remains whether these new kinase targeting approaches work.

Synthetic Lethality

Another possible approach to the future development of cancer-specific and selectively targeted drugs involves the concept of synthetic lethality. In brief, any two genes may be said to be synthetically lethal if the mutation of either one alone promotes cell survival and the mutation of both leads to cell death. In theory, targeting a synthetically lethal gene to a relevant cancer-related mutation should spare normal cells and kill only cancer cells. This approach has become much more feasible now that chemical and genetic tools for perturbing gene function in somatic cells allow the systematic identification and screening of synthetic lethal genes and their targeting in tumors of interest [Kaelin, 2005].

Systems Biology

Yet another alternative drug discovery approach would be to target the complex systems biology underlying a disease [Ideker et al., 2001]. This approach might be more successful for identifying novel therapeutic targets [Fishman and Porter, 2005]. Drug discovery based on systems biology would concern the behavior and relationships of all the elements in a particular functioning biological system rather than individual genes or proteins considered one at a time. In theory, a greater understanding of the disease network could reveal whether inhibiting a single target would be sufficient to restore the system to a healthy state and, if not sufficient, whether modulating the activity of multiple targets might be required to achieve optimal therapeutic benefit [Keith et al., 2005; Mencher and Wang, 2005]. The different levels of information to be gathered about genes, mRNAs, proteins, and signaling pathways can be integrated, graphically displayed, and then computationally modeled to generate predictive mathematical models of the system. While one can assume that a biological system operating at multiple organizational and hierarchical levels and processing data through a complex network of communication and signaling will not be affected detrimentally by a single targeted perturbation, one can also assume that such a network will contain multiple key nodes that may indeed be profoundly affected by such perturbations. The goal of systems biology is to identify these powerful nodal targets so as to

better understand and then manipulate the disease [Ideker et al., 2001].

Promiscuous Drugs

Drug selectivity is a virtue when a highly selective drug can target a single molecule responsible for the etiology of a disease. Most diseases, however, involve multiple molecular abnormalities. A disease may have more than one dysfunctional protein, and these may be out of balance with each other. A drug may strongly target identical active domains on two different proteins, thereby potentially and simultaneously influencing multiple cellular pathways. Hence, drugs whose efficacy is based on rebalancing the several proteins or events that contribute to the etiology, pathogenesis, and progression of a disease (so-called "promiscuous" drugs) may be ideal. Two such promiscuous drugs developed are sunitinib (Sutent, formerly known as SU11248; Pfizer) for targeting VEGF, PDGF, KIT, and FLT3 receptor tyrosine kinase; sorafenib (BAY 43-9006; Bayer) for targeting VEGF, PDGF, and RAF/MEK/ERK; and NTI-2001 (Natrogen Therapeutics) for modulating several cytokines including TNF-α, IL-1β, IL-6, IL-10, and cyclin D kinases involved in cellular transformation and proliferation (for references, see [Mencher and Wang, 2005]).

Traditional Medicine/Natural Products

Between 1981 and 2002, 48 of 65 drugs approved for cancer treatment were natural products, based on natural products, or mimicked natural products in one form or another [Newman et al., 2003]. Several population-based studies indicate that people in Southeast Asian countries have a much lower risk of developing colon, gastrointestinal, prostate, breast, and other cancers than do their Western counterparts. It is likely that dietary constituents (e.g., garlic, ginger, soybeans, curcumin, onion, tomatoes, cruciferous vegetables, chilies, and green tea) play an important role in protection from these cancers. These dietary agents are believed to suppress the transformative, hyperproliferative, and inflammatory processes that initiate carcinogenesis. Tumor cells use multiple cell survival pathways to prevail [Aggarwal, 2004], and agents that can suppress these multiple pathways have great potential in the treatment of cancer [Aggarwal and Shishodia, 2006]. The molecular targets of chemopreventive agents such as curcumin [Aggarwal et al., 2003], resveratrol [Aggarwal et al., 2004], guggulsterone [Shishodia and Aggarwal, 2004], silymarin [Agarwal et al., 2006], and indole-3-carbinol [Aggarwal and Ichikawa, 2005] are similar to those currently being used to treat cancer. The evidence indicates that most of the plant-based agents used in traditional Ayurvedic and Chinese medicine do indeed suppress multiple pathways (e.g., those of NF-κB, AP-1, JNK, COX-2, cyclin D1, matrix metalloproteinases, iNOS, HER2, EGFR, bcl-2, bcl-X_L, and TNF) that have been implicated in tumorigenesis (Fig. 2). Because of their pharmacological safety, these agents can be used alone or as adjuncts to current chemotherapeutic agents to enhance therapeutic effects and minimize chemotherapy-induced toxicity. Because cancer is primarily a disease of older age, finding less toxic therapies is a major priority. It is estimated that more than 80% of the world's population cannot afford modern medicines. In addition to cost, current cancer therapies are minimally effective and exhibit toxicities that are intolerable in most cases. Hence, the real key to drug discovery lies in the development of therapeutic agents that are safe, effective, and affordable.

CONCLUSION

Advances in molecular biology and high-throughput screening techniques have enabled a major paradigm shift from traditional physiology-based to target-specific drug discovery and development. Nevertheless, this has not necessarily resulted in safer, more effective, or less expensive drugs. In most cases, the cost of taking a single drug from discovery to testing to market is approximately $1 billion. Moreover, most new target-specific drugs are eventually withdrawn, leading to financial losses and expensive product litigation that can fuel the higher cost of future products as makers try to recoup those costs. In reality, there has been no true progress in the arena of drug development. Most target-specific drugs have failed to deliver the expected results, and the basic hypothesis that a single drug can cure cancer has come into question. Consequently, the emphasis in drug discovery and development has begun shifting once again, this time toward multi-targeted therapies involving combinations of drugs and toward the systemic discovery of multi-component therapeutics.

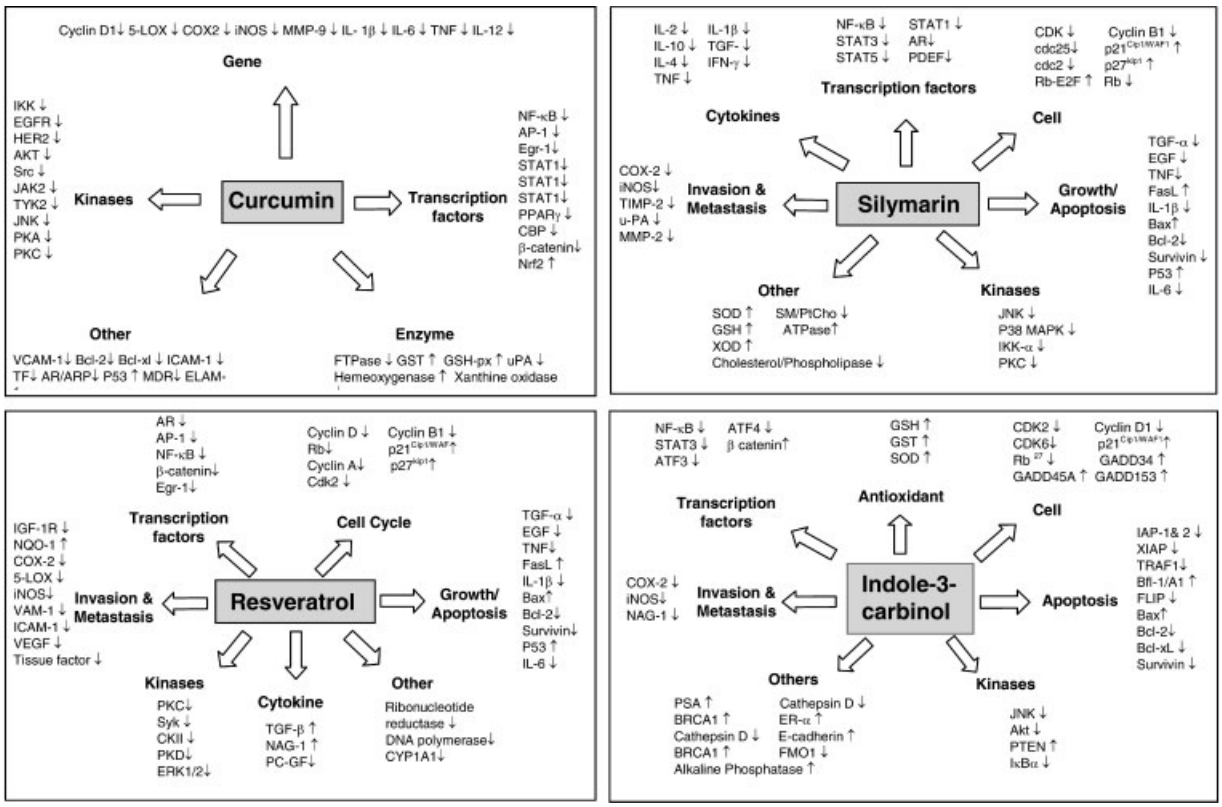

Fig. 2. Molecular targets of curcumin, reveratrol, silymarin, and indole-3 carbinol.

This shifting scene leads us to ask again whether medicine is moving forward or backward on the road to drug discovery. Modern medicine by itself continues to fall short of the expectations of both patients and clinicians and its much-touted promise to cure multifactorial diseases through targeted therapies. As a result, much more attention is now being paid to traditional and integrative medicine. One might even wonder whether the new key to drug discovery lies not in targeting specific signaling pathways but in holistic systems biology. In any case, there is an urgent and serious need to reconsider the wisdom of continuing on a path (i.e., single-target drug discovery and development) that may never lead to safe, effective, and affordable anticancer drugs.

ACKNOWLEDGMENTS

We would like to thank ChaRhonda Chilton for a careful review of the manuscript. This work was supported by funds from the Clayton Foundation for Research (to BBA), and an NCI Cancer Center Core Grant (CA-16672) (to M. D. Anderson Cancer Center).

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