Mapping a route to success

Kinase mutation mapping can be used to pinpoint responder populations.

BY KRISTA STEGER AND PAUL McEWAN

Pharmacogenomics offers patients the hope of personalized medicine—the ability to have drugs and therapies selected for the highest efficacy and the fewest side effects, all based on specific genetic makeup. The realization of personalized medicine, however, may be years away. Genomic variants must first be identified and correlated with specific outcomes such as disease predisposition, drug responsiveness, or adverse side effects. Additionally, diagnostic assays to detect these genetic biomarkers must be developed for use in patient screening and introduced into the health care system.

In contrast, pharmaceutical companies are in a position to benefit from the field of pharmacogenomics right now. Pharmacogenomic studies often shed light on the molecular mechanism of disease and drug inhibition not seen using other avenues of research. Moreover, information gathered from previous pharmacogenomic studies can be incorporated during preclinical R&D to rationally develop drugs with an increased likelihood of success. Companies can use patient polymorphism stratification to subclassify disease types or to identify responder populations and increase clinical trial success. Additionally, information regarding the mechanism of drug or chemotherapeutic resistance is often gleaned using pharmacogenomics, which allows companies to develop efficacious second-line treatments. Candidate drugs showing poor clinical results may be rescued by identifying low-frequency responder populations.

These applications have both immediate and long-term financial ramifications for companies by enhancing the speed and decreasing the costs of drug development. Current industry activities suggest a trend toward incorporating pharmacogenomics earlier in drug development pipelines.

An initial application of pharmacogenomics was the identification of polymorphisms in genes encoding drug-metabolizing enzymes, such as cytochrome P450, and the characterization of their correlation with drug efficacy and toxicity. More recently, investigators have begun to examine polymorphisms and mutations within genes either known or suspected to be molecular targets of candidate drugs.

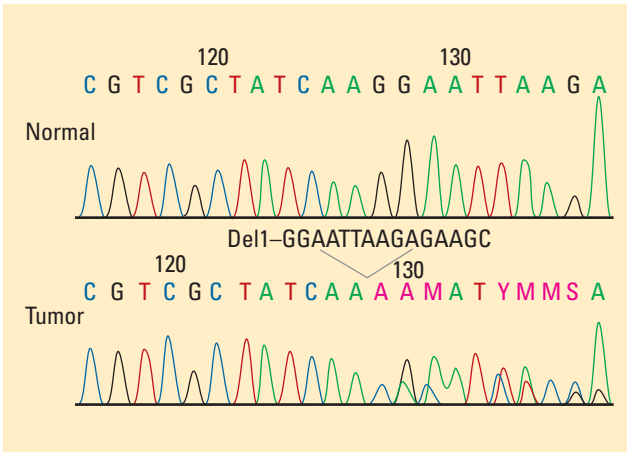

Figure 1. Deletion detected within the kinase domain of the EGFR gene in gefitinib-responsive patients with non-small cell lung cancer.

The story of kinases

Kinases are central players in normal and diseased cell biology. Accordingly, many newly approved and late-stage drug candidates target kinases and kinase signaling pathways. One such class of pharmaceuticals, tyrosine kinase inhibitors (TKIs), has already proven to be efficacious in treating a number of human diseases. Recent clinical and scientific observations of kinase involvement in disease and the correlation of specific kinase mutations to drug responsiveness have brought TKIs into the spotlight. There are several lessons that can be learned from early TKI experiences with drug resistance and poor clinical trials.

Gleevec (imatinib mesylate), developed by Novartis, was the first true targeted anticancer therapy. It is used in treating chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GISTs), both of which have known alterations in genes encoding for tyrosine kinases.

CML is caused by a specific genetic mutation resulting from a translocation between the long arms of chromosomes 9 and 22. This translocation creates the small Philadelphia (Ph) chromosome that encodes for a fusion protein of Bcr and Abl, a tyrosine kinase. The Bcr-Abl fusion protein has constitutive Abl kinase activity causing uncontrolled cell growth. Gleevec inhibits Bcr-Abl activity, leading to substantially increased survival in CML patients. A second molecular target of Gleevec is c-Kit, a receptor tyrosine kinase known to have a gain-of-function mutation in GISTs.

A mutation (T351I) in the Abl kinase domain of the Bcr-Abl gene was recently identified that produces a fusion protein that is over 200-fold less sensitive to Gleevec (*1*). This detailed information on the mechanism of drug resistance allows for rational design, rather than more prolonged trial-and-error-based development, of second-line compounds that can inhibit Gleevec-resistant tumors. Not only is this speeding the development of alternative therapeutics, but using compound analogs also allows companies to capitalize on previous original development research.

Epidermal growth factor receptor (EGFR) is unregulated in a wide range of cancer types. Preclinical studies have demonstrated that compounds correcting EGFR abnormalities exhibit antitumor activity. On the basis of these findings, therapeutic strategies to inhibit EGFR or EGFR-related pathways are prime molecular targets in drug discovery pipelines.

AstraZeneca's Iressa (gefitinib) is one of many small-molecule drugs directed specifically against EGFR. While Japanese trials of gefitinib were promising, results from two large, U.S.-based trials showed no survival benefit by adding it to standard chemotherapy as an initial treatment for advanced non-small cell lung cancer (NSCLC). Overall, the response rate to gefitinib was only 10%, although there were clearly more dramatic responses in some patients. The lack of survival benefit and the low response rates were cause enough to cancel other late-stage trials, although on the basis of the response rate, the FDA granted accelerated approval to the drug in May 2003.

Two independent publications released in April from scientists at the Dana Farber Cancer Institute and Massachusetts General Hospital described groundbreaking studies that linked mutations in the EGFR gene to NSCLC patient response to gefitinib (*2, 3*). Both studies used gene resequencing to identify somatic mutations, including a point mutation and two deletions in the kinase domain of the EGFR gene (Figure 1) in tumors from gefitinib-responsive patients. As a result, gefitinib has gained renewed interest as an NSCLC treatment, because a specific responder subpopulation can be identified. These studies also afford AstraZeneca the opportunity to rationally design gefitinib analogs with increased inhibitory activity against wild-type EGFR.

Avenue of resequencing

Public databases containing known single nucleotide polymorphisms (SNPs) continue to be useful tools for designing genetic association studies. However, these databases are incomplete in any particular genomic region, and deeper sampling is often required to identify causative mutations. For most drugs in development, the genetic variations that determine their clinical response remain uncovered. Therefore, detailed polymorphism mapping studies are most often required prior to studies to correlate specific genomic biomarkers with drug responsiveness or disease susceptibility.

There is a plethora of high-throughput screening assays for analyzing kinase inhibition at the protein level. These are critical in the initial phases of R&D to identify potential candidate drugs, but they are not suitable readouts for detecting genomic variation. Additionally, a wide array of technologies are available for use in SNP genotyping, including, but certainly not limited to, mass spectroscopy, real-time PCR and melting curve analysis, single-base extension assays, and fluorescence plate-based assays. One central caveat of these genotyping assays is the need for previous knowledge of the mutations to be assessed. Therefore, while these technologies are useful in high-throughput patient screening, they are not applicable to discovering genomic biomarkers.

The gold standard for identifying polymorphisms in genes of interest is resequencing. It produces high-quality, high-resolution maps not only of SNPs but also of deletions and insertions that are common abnormalities in cancer cells. Resequencing begins with gene modeling, in which primer sets are designed to tile the length of the gene of interest. This allows for the PCR amplification of short 500–600-bp fragments called amplicons, which are easily sequenced. In silico modeling software speeds the design process and can optimize primer sequences to increase amplicon success rates. It remains critical, however, that each amplicon in the gene be wet-lab-validated to ensure production of a single amplicon with a clean sequence. If necessary, secondary primer sets must be designed. If wet-lab validation is not conducted, needless time and money are spent screening samples.

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SNP Discovery and Resequencing services from Agencourt Bioscience are one option tailored for identifying gene polymorphisms using proprietary amplicon modeling followed by full wet-lab validation and optimization. The company has a high-throughput sequencing pipeline integrated by a customized laboratory information management system and data analysis software to produce the high-quality polymorphism maps needed for subsequent correlative patient studies. Another unique feature of the system is an extensive polymorphism-calling process. It combines automated and manual review of all polymorphisms to enable highly accurate and complete identification of both SNPs and insertions and deletions, which are often miscalled by automated software packages.

Moving forward

Any major pharmaceutical development pipeline inevitably contains a tyrosine kinase inhibitor. EGFR is just one area of focus. Tyrosine kinases involved in angiogenesis—such as Flt-3 and the receptors for VEGF, FGF, and PDGF—are a second area of concentration. These anti-angiogenic compounds have shown promise as possible anticancer therapeutics.

Realizing the growing need for kinase polymorphism screening, Agencourt now offers a mutation mapping service based on a large set of prevalidated kinase assays. The assay panel represents over 93 kinases, oncogenes, and proto-oncogenes, including EGFR, most of which have been licensed from the Dana Farber Cancer Institute. These assays have all been modeled, fully validated, and used in mapping studies.

"We felt it was important to make these assays available to all scientists to speed up the drug discovery process," says Lynn Doucette-Stamm, vice president of business development at Agencourt. This unique program provides an opportunity for anyone doing basic R&D, as well as preclinical or clinical trials, to quickly map genes of interest without sacrificing the needed quality.

It is an exciting time in the field of pharmacogenomics. As more detailed mutational mapping information becomes available, the closer we become to identifying genetic biomarkers and screening assays to make personalized medicine a reality.

References

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