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Nicholas C. Dracopoli

Pharmacogenomic applications in clinical drug development

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Abstract Pharmacogenomics is being increasingly applied to the development of novel oncology drugs. Single nucleotide polymorphism genotyping of known drug-metabolizing and transport genes is used to screen for pharmacokinetic differences in drug exposure, and genome-wide transcription profiling is being widely used to identify expression profiles that can be correlated to pharmacodynamic variables resulting from molecular changes within tumors. Given the relatively low efficacy of most cancer therapies, and the molecular heterogeneity within tumors, pharmacogenomics provides one of the best opportunities for improvements in drug efficacy through effective stratification of patients, and early identification of those individuals most likely to respond effectively to a specific therapeutic approach.

Keywords Pharmacogenomics · Pharmacogenetics · Drug discovery · Transcription profiling

Introduction

Pharmacogenomics can be defined as the use of biological markers (DNA, RNA, or protein) to predict the efficacy of a drug and the likelihood of the occurrence of an adverse event in individual patients [5]. A major goal for cancer pharmacogenomics is to build on the standard clinical and pathological classification of cancers by tissue of origin, stage, and grade by defining the individual molecular causes of malignancy in each tumor. Cancer arises from a limited number of changes to specified regulatory pathways (e.g. cell-cycle regulation, signal transduction, and apoptosis). Therefore it is possible that genome-wide expression scanning may reveal a small number of signatures that can be used to define molecular profiles of specific malignancies that can be correlated with drug response and disease outcome to search for markers of drug response.

We have used transcriptional profiling of several thousand genes in tumor cell lines, xenografts, and human tumor samples to scan for differences in patterns of gene expression. Using current transcriptional profiling technology, it is possible to screen all known human genes or expressed sequence tags ($>30{,}000$ putative expressed sequences) using RNA derived from relatively small samples. Therefore it is no longer necessary to form a biological hypothesis to select candidate genes for screening, but it is possible to screen all known genes to identify a small subset of gene expression markers that define molecular subclasses correlated with treatment response and disease outcome. Using these approaches, it is possible to develop molecular profiles of solid tumors, and to use these profiles to predict treatment response and outcome. When validated, these data could be used to develop pharmacogenomic tests that will provide important guidance in the selection of optimal therapeutic approaches for each individual. We expect that these approaches will become common practice and eventually be widely used in the development of new, more effective approaches to the treatment of cancer.

Impact of genomics on pharmaceutical discovery and development

The completion of mapping the human genome sequence and development of high-throughput genomic technologies is having a profound impact on the pharmaceutical industry. This impact will be felt at all stages

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N. C. Dracopoli
Bristol-Myers Squibb, Route 206 and Provinceline Road,
Princeton, NJ 08540, USA
E-mail: nicholas.dracopoli@bms.com
Tel.: +1-609-2524527
Fax: +1-609-2526313

of the drug development process. In particular, the identification of the approximately 30,000 human genes will dramatically increase the numbers of targets (including G-protein-coupled receptors, kinases, and proteases) that are amenable to treatment with small molecules, as well as identifying novel target families that have not previously been available for small-molecule drug development.

The discovery of comprehensive lists of single nucleotide polymorphisms (SNPs) [7] has significantly improved our ability to scan the genome for the molecular causes of disease, as well as for the interindividual differences that underlie variation in drug response and risk of adverse events. New technologies will soon allow rapid and relatively inexpensive genome scanning that will lead to the identification of novel complex disease genes as new drug targets, and markers for disease progression and drug response that will be used to guide therapeutic decisions. These technologies are key to the development of personalized medicine, for selection of therapeutic agents and drug dose, as well as for the eventual discovery of risk markers for use in preventative medicine.

Pharmacogenomic technologies

There is a broad range of technologies that can be applied to pharmacogenomics. These technologies fall into two basic categories. Firstly, profiling technologies including mass spectrometry (MS) for proteomics, expression profiling, and genotyping are used to identify profiles of large numbers of markers that can be mined for association with defined biological endpoints. Secondly, standard analytical methods including immunoassays, flow cytometry, and MS are used to deliver validated biological assays in the clinic.

Profiling experiments are carried out on a small number of samples and involve the analysis of hundreds or thousands of markers. For example, a proteomics experiment may attempt to resolve complex mixtures of up to 2500 proteins using tandem MS-based methods, a genotyping experiment may look at hundreds or even thousands of SNPs in selected candidate genes and eventually be able to scan the whole genome [6]. In contrast, a transcription profiling experiment may look at all the approximately 30,000 known genes using microarrays or gene chip-based methods. The profiles from these experiments are then analyzed to search for associations with defined biological endpoints (drug response, disease status, time to disease progression, etc.), and a small number of candidate markers identified. These markers then need to be clinically validated by analysis of an independent set of samples, and reduced to the minimum set that provides the maximum correlation with the desired endpoint.

The identification of pharmacogenomic markers is essentially a pattern recognition problem. The patterns of gene expression, SNP genotypes, peptide mass spectra, or high-field nuclear magnetic resonance spectra for small metabolites are collected for samples with the desired biological endpoints. For example, tumor samples can be analyzed from patients who are sensitive or resistant to a particular therapy. These profiles are then scanned to search for patterns that correlate with the selected biological endpoints and used to distinguish between them. Many tools have been developed for these analyses, including both supervised and unsupervised clustering methods to identify sets of markers correlated with the desired endpoints [1, 2, 9].

The validated markers need to be converted into assays that can be delivered efficiently and cost-effectively in the clinic. Profiling technologies are research tools that are not suitable for widespread clinical analysis. Also, routine pathological procedures do not preserve tissue samples appropriately (e.g. routine collection of frozen tumor biopsies for preservation of RNA), so it is necessary to convert the profiling markers into assay formats using standard clinical laboratory equipment. This will often require the development of antibodies and probes that can be used to measure protein or RNA levels using immunologically based or hybridization-based methodologies.

Cancer pharmacogenomics

Oncology is a key area for the development of pharmacogenomics. The typically low efficacy rate (<30%) of most cytotoxic and targeted cancer therapies provides a great opportunity for increasing drug treatment efficacy by selecting the patients most likely to respond to therapy. The genetic variability present in tumors also provides a unique source of markers that can be used to correlate disease outcome and risk of adverse events. The currently marketed compound, trastuzumab, already sets a precedent for the use of pharmacogenomics to guide clinical decisions in selection of optimal therapy for breast cancer [8].

Variability in drug response can result from either pharmacokinetic or pharmacodynamic differences between patients. Pharmacokinetic variability results from differences in drug metabolism or drug transport that determine the relative exposure of the target tissue to different therapeutic compounds. Individuals with mutations in these genes may have either very different exposures to the same dose of a drug resulting from impairment or acceleration of the drug metabolism or efflux, and have to be treated with correspondingly different doses to obtain the same therapeutic effect. For example, variability in the thiopurine S-methyltransferase gene causes rare individuals to be slow metabolizers of mercaptopurine, a compound used in the treatment of childhood leukemia. Slow metabolizers of mercaptopurine are at high risk of a serious adverse event unless treated with a significantly lower dose [4].

Most recent cancer pharmacogenomic profiling experiments have focused on pharmacodynamic differences

between tumors. The model for these experiments assumes that there are a limited number of changes that occur during the transformation of a normal epithelial cell into an advanced malignancy. If there were too many changes, no detectable predictive pattern would be expected to emerge from the analysis of small sets of tumors. It has been demonstrated that cancer arises from a limited number of genetic changes in essential regulatory pathways (e.g. cell-cycle regulation, signal transduction, and apoptosis) [3]. While there is a vast heterogeneity of different mutations, deletions, and amplifications during tumor development, the hope for these transcriptional profiling experiments is that the abrogation of any particular pathway would lead to a specific pattern of expression of genes located downstream of the mutational event. If this is the case, it should be possible to detect gene expression "signatures" that reflect alterations in specific regulatory pathways, which can be correlated with disease outcome or drug response to identify novel pharmacogenomic markers.

The timing of such mutational events during tumor progression is another complicating issue. Genome-wide transcriptional profiling experiments are carried out on RNA extracted from tumor biopsy samples. These experiments presume that the genetic changes that determine the ultimate fate of the tumor are present at the time of the biopsy in a sufficient proportion of the tumor to allow for the detection of the profiling signal.

A variety of studies have been completed using these approaches to make both retrospective predictions of efficacy for existing chemotherapeutic agents [10], as well as for prospective studies of compounds during drug development. While retrospective studies can have a major impact on the utilization of existing chemotherapeutic agents, the most benefit is likely to be achieved through prospective studies of drugs in development. The combination of analyses of preclinical models (cell lines and xenografts) with early clinical studies can help stratify patients in clinical trials, and determine optimal indications for compounds in early development.

Preclinical studies will be key to developing effective clinical development strategies for novel compounds. They can be completed long before the "first-in-human" studies, and can be useful in guiding phase II and subsequent trials. Two basic preclinical strategies have been followed. One method is to complete profiling on a panel of cell lines and compare expression patterns with the drug response data. For example, Weinstein et al. [10] have calculated the IC_{50} (drug dose that reduces cell growth by 50%) for a large number of compounds on a standard panel of tumor cell lines. These data have then been analyzed to search the expression profiles for markers whose expression pattern correlates with individual compounds or groups of compounds with similar chemotypes. A second approach to preclinical studies is to complete dose-response experiments, and look for markers whose expression correlates with subapoptotic doses of the experimental compounds.

In one analysis of a novel compound being developed at Bristol-Myers Squibb, both these preclinical approaches have been used to identify a common set of markers by comparing these types of experiments. The 25 genes that were most consistently overexpressed in drug-sensitive cells were compared with 51 genes that were downregulated after treatment with this novel compound. A total of six genes were contained in this overlap ($P < 10^{-8}$) between the two experiments. Interestingly, two of these six genes were known to be in the downstream signaling pathway of the target for this novel compound. This suggests that these coregulated genes can be used as markers of response to this compound. Subsequent analysis of untreated tumor biopsies showed that approximately 30% of tumors have these six genes coordinately overexpressed. Consequently, if the tumors behave in vivo as they have been shown to do in vitro, they could be used as markers to stratify patients in clinical trials. If this proves to be the case, it may be possible to increase the efficacy of this compound more than threefold by preselection of patients who are most likely to respond to this novel therapy.

Pharmacogenomics is being widely applied to cancer drug development, as this may represent the best opportunity for increasing efficacy for newly developed targeted therapeutics, and for the best definition of areas of unmet medical need that can be recycled back for further drug discovery. The successful application of these approaches could eventually lead to a reclassification of cancer. Instead of being defined by tissue of origin and tumor stage and grade (e.g. stage IIIb breast cancer), tumors will have a molecular pathology including expression profile and lists of mutated oncogenes and deleted suppressor genes added to the existing pathological analyses.

Summary

Pharmacogenomics will have an increasing impact on drug development over the next few years. The first pharmacogenomic products have already reached the market and will soon be joined by other products where the pharmacogenomic marker is the status of the drug target (as is the case with trastuzumab), and eventually by pharmacogenomic markers developed from genome-wide profiling technologies. Pharmacogenomics will be likely to have an impact on productivity through patient stratification leading to smaller, faster, and less-expensive clinical trials. Pharmacogenomic approaches will also be used to improve the selection of drug targets and to identify novel areas of unmet medical need for future discovery research. More controversially, pharmacogenomics may be used to "rescue" drugs in late development that have either low efficacy against the total disease population, or by identifying patients at high risk of adverse events.

While reduction in the risk of adverse events is an enticing proposition, it remains to be shown whether it is possible to identify sets of markers with sufficient specificity and sensitivity for complex conditions (such as hypersensitivity or edema), that could significantly reduce the frequency of adverse events. Despite this, it is clear that pharmacogenomics will be widely used to predict drug efficacy in cases where monitoring the drug response is difficult (not blood pressure or lipid levels, for example) and where there are important clinical decisions to be made based on the results of these tests. Clearly, pharmacoeconomics will have an important impact on the development of any individual pharmacogenomic marker. Screening for rare events will always be expensive because of the low yield for the tests, and will only be justifiable where there is significant medical benefit to be gained from the test. Consequently, tests for rare adverse events will only be practical for medically important drugs where the adverse events are both rare and very serious.

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