Genetic Testing in Cancer Therapeutics

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A major challenge to physicians administering drugs with a low therapeutic index, such as chemotherapeutic agents, is the variability in drug disposition and clearance. This variability accounts for the wide interpatient response to conventional doses of antineoplastic agents. Currently, most dosing regimens are based on body weight or surface area, with the decision for dose modifications typically being made after the appearance of toxicity. Furthermore, the use of these potentially toxic agents with individuals who are unlikely to receive any benefit makes the blind use of these drugs undesirable. The goal, thus, becomes to select the drug that will likely be most efficacious in an individual patient (see Fig. 1), emphasizing the need for better methods of predicting outcome and response to treatment (1).

Over the past two to three decades, clinical cancer researchers have shown an increasing interest in the role of genetic pathways encoding drug-metabolizing enzymes. The main focus has been on developing genetic tests for abnormalities in pathways potentially influencing response to treatment, particularly inherited polymorphisms affecting drug metabolism and disposition (2–4). The interest in predicting response to treatment or reducing the risk of an adverse drug reaction (ADR) has been driven by pharmacogenetic investigations linking an individual’s DNA blueprint with drug toxicity and more recently efficacy. These pharmacogenomic studies have suggested that response to treatment is, in essence, genetically associated and could be predicted by a phenotypic/genotypic test.

To date, the effect of pharmacogenetic testing on drug response and ADR remains modest. This is despite the fact that ADR ranks between the 4th and 6th cause of death in the United States for all therapeutic agents (5). For safety reasons, several drugs have been withdrawn from the U.S. market in the past several years (6). Annually, more than two million hospitalized patients suffer severe ADR, even following the administration of conventional (standard) doses of chemotherapy, in patients receiving conventional doses (5, 7). Opportunities to reduce or prevent ADR or toxicity due to genetic variations in drug-metabolizing enzyme(s) are now possible as a result of increased genetic knowledge from the Human Genome Project and other pharmacogenetic studies (8, 9).

Pharmacogenetic approaches in cancer therapeutics aim at identifying patients at risk of developing severe toxicity before the administration of a chemotherapeutic agent. This approach should ultimately allow individualizing therapy through tailored dosing or using treatment modification strategies, thereby avoiding genetically altered drug metabolic pathways. In a systematic review by Phillips et al. (7), it was suggested that in >50% of drugs examined in ADR studies, genetic variability, at least in one of the enzymes associated with altered metabolism, was responsible for the development of such ADR.

Pharmacogenetics and Cancer Therapeutics

Currently, research laboratories in academia and industry have been attempting to develop phenotypic and/or genotypic tests to rapidly identify and screen for susceptibility and/or response-conferring genotypes. Pharmacogenetic studies in the cancer therapeutics area have shown an association between specific genetic variants of drug-metabolizing enzymes (pharmacogenetic determinants of response) and ADR or toxicity. One of the difficulties in discussing this topic is the nomenclature used to describe similar entities in the literature. Previously used nomenclature in the scientific literature has been modified to accommodate subtle differences and to more precisely describe the specific topic (e.g., pharmacogenetics/pharmacogenomics; ADR/adverse drug event).

Genetic variations in phase I drug-metabolizing enzymes, such as CYP3A4 and CYP3A5, have been associated with altered responses of patients to conventional doses of chemotherapeutic agents, such as etoposide (10), teniposide, paclitaxel, and vincristine (11). Of note, however, is that dose modifications of these drugs are not routinely used for individuals with altered CYP3A4 and CYP3A5. In December 2004, the Food and Drug Administration (FDA) approved the first genotyping test for the evaluation of the metabolizer status of patients.1 This test detects genetic variations in the cytochrome P450 (CYP) enzyme (CYP2D6) through a microarray AmpliChip Cytchrome P450 (Roche, Indianapolis, IN) diagnostic device, classified by the FDA as a drug-metabolizing enzyme genotyping system (class II). Although valuable information is provided by the AmpliChip Cytochrome P450 genotyping test, it is not intended to be a stand alone test (12) and should be coupled with clinical evaluation and measures of response to determine the optimum dose and best treatment options for each patient.

Genetic alterations in phase II drug-metabolizing enzymes affecting cancer chemotherapy agents, including thiopurine S-methyltransferase (TPMT), has been associated with hematopoietic toxicity, in patients receiving conventional doses

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of 6-mercaptopurine. Different alleles of UDP-glucuronosyltransferase (UGT1A1) have been associated with severe life-threatening diarrhea and neutropenia in patients receiving conventional doses of irinotecan, and altered N-acetyl transferase has been associated with myelotoxicity in patients receiving standard doses of the experimental agent amonafide (11).

Dihydropyrimidine dehydrogenase (DPD) deficiency caused by inactivating mutations (e.g., DPYD*2A mutation) in the DPYD gene has been associated with 5-fluorouracil (5-FU) toxicity in 30% to 40% of DPD-deficient patients (13).

Altered alleles of drug transporters, P-glycoprotein (multidrug resistance 1) and multidrug resistance–related protein, have been also associated with interpatient pharmacokinetic variability and anticancer drug resistance. Altered drug targets, 5,10-methylenetetrahydrofolate reductase and thymidylate synthase, have been also associated with 5-FU and antifolate toxicity and drug resistance (11). Several of these examples are described in further detail in this article.

**Pharmacogenetic Determinants of Response: Regulatory Concerns**

Until recently, the state of genetic testing, or more precisely, the state of pharmacogenetic determinants of response to chemotherapy has not been fully FDA regulated, with the exception of the recently approved labeling for TPMT and UGT1A1 genetic testing. Previously, the NIH/Department of Energy Working Group on Ethical, Legal, and Social Implications of Human Genome Research convened a Task Force on Genetic Testing to investigate the state of genetic testing. The Task Force raised several concerns on the process of genetic testing, specifically the clinical and analytic validity of these tests. The Task Force indicated that a protocol for genetic test development would be limited in the absence of appropriate data pertaining to analytic and clinical validity, significance, and usefulness of predictive tests and measures of laboratory quality and reimbursement of laboratory test costs by the various health insurance providers (14–16). The increasing number of genetic tests, whether provided by nonprofit organizations, requiring only Institutional Review Board approval and/or Clinical Laboratory Improvement Act certification (not regulated by FDA) or by biotechnology companies as diagnostic kits (regulated by FDA), has gradually elevated the awareness and concerns regarding the information provided to practitioners or patients by biotechnology companies and nonprofit organizations (17, 18). A survey reviewing 115 drug labels (17, 18) has shown that only 3 of 10 concerns were noted in the majority of these labels (which candidates are appropriate for testing, which clinical conditions require testing, and whether genetic counseling was needed). Risks, limitations, and benefits of genetic testing were described in ~10% of drug labels. Confidentiality and informed consent.
were referred to in <30% of drug labels. Test performance (sensitivity, specificity, and predictive value) described in ~50% of drug labels has been vaguely referred to as accuracy (17, 18) without definite measures of performance variables. Nonprofit organizations were more likely to state patient rights and need for counseling in their drug labels, whereas biotechnology companies were more likely to specify the purpose of the test (14, 17). Taken collectively, the Task Force recommended the FDA to consider new legislative policies to be implemented in the development and approval of genetic tests (15, 16).

FDA Efforts in Regulating Genomic Biomarkers of Response

The recent FDA approval of UGT1A1 pharmacogenetic testing for irinotecan chemotherapy followed a strategic plan (19) aimed at developing standards of specialized genetic testing methodologies (pharmacogenetics) to provide a safe effective treatment. In July 2004, a workshop was held to identify issues in the development of “drug test combination products” (20, 21), and a concept article was published (21). At the present time, FDA regulations require the description of data pertaining to genomic biomarkers in both full and abbreviated reports. Currently, the type of genomic data to be reviewed in an New Drug Application submission depends on the purpose of the genomic evaluation and the validity of the genomic biomarker (14–16).

Pharmacogenetic Testing for TPMT

The thiopurine drugs 6-mercaptopurine, 6-thioguanine, and azathioprine are prodrugs used in the treatment of acute lymphoblastic leukemia, autoimmune disorders, and inflammatory bowel disease. Efficacy and cytotoxicity of these prodrugs is achieved through metabolic conversion into active 6-thioguanine nucleotides. TPMT catalyzes the S-methylation of these thiopurine prodrugs into inactive metabolites. Thiopurine drug toxicity has been associated with low TPMT activity inherited as an autosomal codominant trait (22). Population studies investigating TPMT polymorphisms in Caucasians, Asians, Africans, and African Americans associated the presence of polymorphic alleles in the open reading frame of the TPMT gene with low enzyme activity due to enhanced degradation of the variant protein (23). Decreased enzyme activity in 80% to 95% of patients has been attributed to three of nine variant alleles: TPMT*2 (G238C), TPMT*3A (G460A and A719G) prevalent in Caucasians, and TPMT*3C (A719G) prevalent in Asian, African, and African-American populations. A trimodal pattern of distribution characterizes erythrocyte TPMT activity (22). In Caucasian populations, >89% of individuals have high activity; 11% with intermediate activity; and 0.3% are profoundly deficient because of nonfunctional homozygous TPMT alleles (24). Studies investigating treatment of homozygous, TPMT-deficient children having acute lymphoblastic leukemia, with conventional doses of thiopurines, indicated that dose-limiting hematopoietic toxicity is more likely to develop, whereas a heterozygous TPMT genotype typically shows intermediate tolerability. Patients with wild-type TPMT typically have better tolerance to 6-mercaptopurine compared with heterozygous and homozygous patients (25, 26). With the recent recognition of the importance of TPMT pharmacogenetics and confirmation of the effect of TPMT alleles on drug disposition, clearance, and risk of toxicity, the FDA approved the implementation of TPMT genetic testing on the labels of thiopurine drugs (22).

Pharmacogenetic Testing for UDG-Glucuronosyltransferases

The use of irinotecan (Camptosar), a cytotoxic agent approved for the treatment of metastatic colorectal cancer, is limited by toxicity, including life-threatening diarrhea and neutropenia most commonly observed with the weekly (27) and the 3-week schedule, respectively. Irinotecan is metabolized to the active form SN-38 by carboxylesterases (28–31). SN-38 is further metabolized by glucuronosyltransferases, mainly UGT1A1, to inactive metabolite(s). A minor fraction of SN-38 is also metabolized by CYP3A4 and CYP3A5. Population studies have associated different UGT1A1 alleles with toxicity to irinotecan. In patients treated with irinotecan (weekly schedule), diarrhea seemed to be a life-threatening toxicity that correlated with decreased glucuronidation, whereas neutropenia (every-3-week schedule) correlated with the UGT1A1*28 genotype that led to a higher exposure to SN-38. In the recently modified label for irinotecan (FDA action date: July 17, 2005), a reduction by at least one level in the starting dose of irinotecan has been suggested for patients homozygous for the UGT1A1*28 allele. UGT1A1*6 was consistently associated with neonatal hyperbilirubinemia in Asians, whereas the association of UGT1A1*60 with irinotecan pharmacokinetics and bilirubin levels has been inconsistent in review of the literature (32, 33). In addition, irinotecan toxicity will be further defined when the roles of drug transporters (e.g., breast cancer resistance protein, multidrug resistance protein 2, and organic anion transporting polypeptide 1B1) in irinotecan disposition and clearance have been further elucidated (1). The recent approval by the FDA of UGT1A1*28 genetic testing using the Invader assay emphasizes the growing interest in individualizing drug therapy. Although the toxicity associated with a homozygous UGT1A1*28 allele has been referred to in the most recently modified irinotecan label (action date: July 17, 2005), there has been no emphasis or clear recommendation on the necessity of screening patients for this mutation before the administration of irinotecan. As noted previously, however, dose reduction based on genotype was suggested. The decision to perform genetic testing before the administration of irinotecan has been left at the discretion of the treating physician, which raises the issue of the importance of pharmacogenomic education for the medical profession.

Pharmacogenetic Testing for DPD: a Future Need

Recent population studies investigating 5-FU-related toxicity and DPD enzyme activity (34–40) indicate that DPD deficiency, whether partial or profound, is associated with 5-FU-related toxicity. Although the percentage of individuals affected by UGT1A1- and TPMT-inactivating mutations is higher in contrast to the percentage of individuals affected by DPD inactivating mutations, DPD deficiency (3-5%) has been more frequently associated with fatal outcomes. It should be
noted that the presence of DPYD-inactivating alleles (e.g., DPYD*2A and DPYD*13; refs. 13, 41) are not solely responsible for DPD deficiency as has been recently suggested. Epigenetic regulation, mainly methylation, has also been implicated in the down-regulation of DPD enzyme activity (42), indicating that genetic and epigenetic mechanisms may act separately or in concert to produce DPD enzyme deficiency and 5-FU-related toxicity. Given the complex nature of pyrimidine metabolism, and the fact that 5-FU-related toxicity occurred in a subset of patients with normal DPD enzyme activity, highlights the possible contribution of other genes, in the catabolic (43–45) and possibly the anabolic pathway, in the etiology of 5-FU-related toxicity. Taken collectively, these data suggest that testing a single allele of a single gene is unlikely to provide a complete explanation for a pharmacogenetic condition, such as 5-FU toxicity. Instead, a more comprehensive approach is needed. Recently, a noninvasive breath test (13C-UraBT) was developed to assess the integrity of the entire pyrimidine catabolic pathway (46). This test has been examined in a population of normal healthy volunteers with variable range of DPD enzyme activity (normal/deficient DPD enzyme activity; refs. 46, 47). A population of cancer patients is currently being evaluated to validate the use of this test as a mean of predicting patients at risk of developing 5-FU-related toxicity. Still, another pharmacogenomic approach using haplotype block analysis and single nucleotide polymorphism tagging of the entire 5-FU metabolic pathway is currently being investigated.

Existing Limitations and Future Prospects

Recently, the medical profession became aware of the limitations that have greatly hindered advances in cancer therapeutics and response to treatment. These limitations include the lack of pharmacogenomic education at medical schools, integration of pharmacogenomic knowledge into clinical practice, and recognition and responsiveness to the effect of pharmacogenomics on healthcare. The continued advances in molecular genetics will permit the development of rapid pretreatment screening methods for the prediction of toxicity risks associated with relevant chemotherapeutic regimens. This should eventually lead to improvement in the effectiveness of current chemotherapeutic agents with improved antitumor efficacy and decreased host toxicity. In the era of personalized medicine, it should become mandatory to implement the recent FDA strategies (21) in drug-device combination using well-designed and well-executed studies to provide credible associations between genetic variants and drug disposition, clearance, and response. This highlights the importance of pharmacogenomics as an integral part of drug labels that will guide drug selection, drug dosing, and determining the patient’s suitability and selection for treatment.

References
