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Predictive Factors for Response and Toxicity in Chemotherapy: Pharmacogenomics

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Recent years have seen marked advances in survival from colorectal cancer. This survival improvement has come from advances in many therapeutic modalities, yet none has been more dramatic than the improvement in the efficacy of drug therapy. Until the mid-1990s, 5-fluorouracil (5-FU) was the only agent known to have significant activity against colorectal cancer. In this 5-FU era, patients with metastatic colorectal cancer could expect to obtain a 3–4 month improvement in survival from 5-FU, increasing median survival from 8 to 12 months.[1] Those with adjuvant chemotherapy could expect a 30% improvement in the risk of cancer recurrence or death when treated with 5-FU monotherapy, increasing disease-free survival from approximately 55% to 67%.[2]

With the approval of irinotecan and oxaliplatin, combination chemotherapy has become the standard of care for both metastatic and adjuvant settings. In patients with advanced disease, the combination of 5-FU with either irinotecan or oxaliplatin essentially doubles response rate, and improves survival to approximately 20 months.[3, 4] The subsequent incorporation of biologic agents targeting vascular endothelial growth factor (bevacizumab) and epidermal growth factor receptor (cetuximab and panitumumab) into therapy of metastatic colorectal cancer has extended median survival to over 2 years[5]—clearly a marked improvement over the 12 month survival afforded by 5-FU monotherapy. The incorporation of oxaliplatin into 5-FU based adjuvant therapy nearly doubles the efficacy of adjuvant therapy, further improving disease-free survival over 5-FU by 23%.[6]

Patient heterogeneity and pharmacogenetics

Thus, these new agents have truly improved the outlook from many patients diagnosed with colorectal cancer. However, not all patients benefit from these drugs. Like many cancers,

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colorectal cancer is heterogeneous: Some patients with stage IV cancer have only one or two liver metastases and will have a fair chance at long-term cancer free survival with a combination approach of surgery with chemotherapy,[7] while others present with bulky liver metastases and die within a year of their diagnosis. Similarly, though modern chemotherapy is increasingly effective, response rates to most regimens hover around 50%, with a substantial minority of patients experiencing disease progression through first-line chemotherapy. In addition to the heterogeneity in tumors and their response to chemotherapy, tolerance of chemotherapy is quite variable. Although many of the combinations regimens are generally well tolerating, allowing many patients to continue to work or carry on their routine during treatment, some patients become exceedingly ill, and even die, from colorectal cancer chemotherapies.

The factors behind these differences in treatment response and tolerance are certainly complex. Inherent differences in cancer susceptibility, perhaps because of differing pathways to carcinogenesis may underlie some of the heterogeneity in tumor response. How an individual cancer patient's system handles chemotherapeutics agents, however, is also an important contributor to inter-patient variability in cancer outcomes. Drug handling, including absorption, distribution, metabolism, and elimination, can be influenced by external factors such as diet, by changes in organ function, and importantly by inherent differences in the activity of drug metabolizing enzymes. Pharmacogenomics is the study of genetic differences in drug handling genes that lead to functional differences is how patients are able to either activate or eliminate drugs.

Pharmacogenetics can help explain some of the inter-patient differences in outcomes, and may allow either dose adjustment based on a patient's a prior risk of toxicity, or may encourage selection of separate drug if a patient's genetic make-up suggests they will be unlikely to respond to or are very likely to have severe toxicity from a given drug.

Technology advances

Though the rationale behind investigating genetic differences in drug handling has been around for many years, it is only recently that the technology for studying genetic differences has allowed for a comprehensive, high-throughput study of this field. Indeed, the changes in the past decade have been dramatic. The efforts of the human genome project delivered not only the sequence of mammalian species, but also stimulated the development of analytical approaches for research and clinical usage. Indeed, one can now purchase genomic systems with which to assess over 1,000,000 unique single nucleotide polymorphisms or perform comprehensive resequencing across large amounts of a person's DNA. The research assays have lead to improved clinical tools, enhancing the ability to apply genomics in practice.

Next, we will discuss three of the most prominent examples of pharmacogenetics that have clinical relevance for the treatment of colorectal cancer patients (Table 1). Though not a comprehensive study, these examples, dipyrimidine dehydrogenase (DPD), thymidylate synthase (TS), UDP-glucuronosyltransferase 1 family polypeptide A1 (UGT1A1), are those with current clinical applicability and are good examples of both the potential of pharmacogenetic study as well as the pitfalls that come when these are brought into routine usage.

Dihydropyrimidine dehydrogenase

The enzyme dyhydropyrimidine dehydrogenase is an excellent example of how a germline differences in a drug elimination enzyme can lead to variable enzyme activity and subsequent differences in chemotherapy tolerance. DPD is the initial, rate limiting enzyme that catabolizes pyrimidines and is responsible for 85% of 5-FU degradation.[8] Deficiencies in DPD resulting in increased 5-FU toxicity have been reported for decades—principally with reports of patients

found to have very low levels of DPD activity after severe, even fatal, toxicity from 5-FU.[9–11] Toxicity in DPD deficient patients is typically a more severe form of common 5-FU toxicity, with severe cytopenias, mucositis, and diarrhea being the most prominent adverse effects, though neurotoxicity can also be a prominent finding. Amongst those with severe (grade 3 or 4) toxicities from 5-FU, as many as 60% may have a baseline deficiency in DPD activity.[12]

After the initial reports associating low DPD activity with 5-FU toxicity, familial studies suggested DPD deficiency appears to be a heritable condition.[10, 11, 13] Based on the distribution of DPD activity in the population, as many as 3% of patients are estimated to be heterozygous for a mutation resulting in decreased DPD enzyme activity, while an estimated 0.1% are homozygous.[13, 14] Over 20 mutations in DPYD that are associated with decreased DPD enzyme activity have been identified, and an additional 10 to 20 single nucleotide polymorphisms or deletions with no known consequence have also been identified.[15] The most commonly found mutation, with an estimated population prevalence of 1%, is a single nucleotide shift from G to A in exon 14. This point mutation, denoted as DPYD*2A, results in a truncated protein with severely limited function,[13] and is found in as many as 40–50% of patients with decreased DPD activity.[12, 16]

With an estimated 3% of the population at risk for severe or fatal toxicity from 5-FU because of DPD deficiency, and a huge number of patients with colorectal cancer as well as breast, gastroesophageal, head and neck and other cancers treated with 5-FU, the number of patients with decreased DPD activity at risk for excessive 5-FU toxicity is substantial. Thus, a test for DPD deficiency that can reliably predict the likelihood of 5-FU toxicity would certainly be clinically useful. The first test for measuring DPD activity tested enzyme function within peripheral blood mononuclear cells. Though this assay may well predict DPD activity and was used in most of the early reports of the association between DPD activity and 5-FU toxicity, so is known to be associated with a clinically meaningful outcome, it is a technically cumbersome test, precluding translation into routine clinical practice. More recently, investigators have looked at tests measuring uracil, as DPD deficiency impedes uracil degradation leading to high blood uracil levels. Both blood tests and breath tests for uracil are available, though have not yet met the standards of reproducibility and normalization necessary for routine clinical use.[15] Given the difficulties in directly measuring DPD activity, a genomic approach to testing for DPD deficiency would seem to overcome many of these problems. Certainly genetic testing is routinely used in many clinical settings (e.g. prenatal diagnosis) and could be used clinically with a turn-around time rapid enough to make the test clinically useful. Yet, DPD deficiency results not just from germline DPYD mutations, rather, epigenetic mechanisms such as promoter methylation may contribute to low DPD activity in patients with no apparent mutation in the DPYD gene.[17] Furthermore, despite the clear increased risk of 5-FU toxicity amongst patients with know genetic variants in the DPD gene, not all patients with such high risk genotypes suffer severe 5-FU toxicity—their risk is certainly higher than the general population of patients exposed to 5-FU, but it is not 100%. In addition, many patients with normal DPD activity suffer severe adverse effects from 5-FU toxicity.[18] As such, preemptively dose reducing 5-FU based on DPD activity or DPYD genotype is not of clear utility.

Thymidylate synthase

The antineoplastic activity of 5-FU is mediated through a number of mechanisms including inhibition of RNA and DNA synthesis by incorporation of fraudulent pyrimidines, and by inhibition of thymidylate synthase (TS), an enzyme required for pyrimidine synthesis.[19] Of these mechanisms, TS inhibition is a prominent component of the cytotoxicity of 5-FU. Patients with cancers expressing higher levels of TS, either protein or mRNA levels, are less likely to respond to 5-FU and have shorter survivals than patients with tumors that have lower levels of

TS expression.[20–22] One particular TS gene polymorphism in the 5' promoter enhancer region (TSER) is known to affect TS expression. This polymorphism is characterized by 28 base-pair repeats in the TSER region, with the increased number of repeats associated with higher levels of TS expression. For example, those with 2 tandem repeats (TSER *2) have lower levels of TS protein and mRNA expression than those with 3 tandem repeats (TSER*3).[23, 24] In turn, colorectal cancer patients homozygous for the TSER*2 alleles appear to be more likely to have a clinical response to 5-FU than those homozygous for the TSER*3 allele (objective response rate 50% vs 9%).[24] Though the evidence is clear that TS expression, regulated in part by the number of tandem repeats in the TSER, is associated with the likelihood of response to 5-FU, stratification by TS genotype has been undertaken in clinical trials, but is not yet in routine use. How to incorporate TS results in an era of combination chemotherapy where 5-FU is rarely given on its own is still unclear. It will likely need to be incorporated with other predictors of response.

UGT1A1

Irinotecan has been available for the treatment of metastatic colorectal cancer since the 1996. In a sentinel paper, irinotecan given with 5-FU and leucovorin markedly improved outcomes compared with those getting 5-FU and leucovorin alone: response rates increased from 21 to 39%, progression free survival increased from 4.3 to 7.0 months, and survival increased from 12.6 to 14.8 months.[4] Toxicity with this IFL regimen, however, was significant. In fact, in subsequent clinical trials that incorporated IFL into first-line treatment of metastatic colorectal cancer (N9741)[25] and adjuvant therapy (CALGB 89803)[26] toxicity was substantial, including early deaths on treatment. These severe treatment related toxicities were predominated by a combination of severe neutropenia in conjunction with diarrhea, with deaths caused by a sepsis-like syndrome.[27] Subsequent regimens that use irinotecan with 5-FU given as a continuous infusion rather than a bolus (FOLFIRI) are much less toxic, with improved efficacy over IFL.[5] However, even in these better tolerated regimens, more than half of patients suffer severe toxicity, making understanding irinotecan pharmacogenetics an important goal.

After IV administration, irinotecan is metabolized to its more active metabolite, SN-38. SN-38 is in turn eliminated by glucuronidation, predominantly performed by UGT1A1. Polymorphisms in the promoter region of UGT1A1 are related to its activity, and thus the overall exposure of SN-38. Persons with wild-type UGT1A1 allele have 6 TA repeats in the promoter region (UGT1A1*1), while those with the less common variant have 7 TA repeats (UGT1A1*28). Patients with two copies of the UGT1A1*1 allele are more effective at glucuronidation of SN-38 than are those with two copies of the UGT1A1*28 allele; thus when given the same dose of irinotecan, the wild-type homozygotes have less exposure to SN-38 than do the *28 homozygotes.[28] Heterozygous patients appear to fall somewhere in between in their ability to inactivate SN-38.

Early studies in small numbers of patients suggested that the UGT1A1*28 homozygotes were at an increased risk of both diarrhea and neutropenia.[29–32] The FDA subsequently asked Pfizer, the maker of irinotecan, to amend the prescribing information to include a recommendation for starting patients homozygous for the *28 allele at a lower dose of irinotecan. Though some studies following this decision have been contradictory, current evidence suggests that patients homozygous for UGT1A1*28 polymorphism are at increased risk of neutropenia, but not diarrhea. Furthermore, this increased risk is most prominent when patients are treated with doses of 180 mg/m2 or greater.[33] When irinotecan is prescribed at lower doses, typically given on a weekly schedule, there is little difference in toxicity between genotypes. FDA-approved testing for UGT1A1 genotype became available in the summer of 2005, though is not yet routinely used in most centers.

Conclusions

The examples of DPD, TS, and UGT1A1 clearly demonstrate proof of principle—that genetic differences is drug handling are indeed responsible for some of the inter-patient variation in treatment response and treatment toxicity. However, these three also show some of the pitfalls of our past endeavors. Currently, none of these are mandatory for use in clinical practice to make treatment decisions, largely because there is too much additional variation that cannot be accounted for by the differing genotypes. The pathways by which drugs exert their anti-cancer effects and the pathways by which they are eliminated are complex. Inter-patient variation in one enzyme in the pathway clearly can affect clinical outcomes, but it is not the whole story. To be able to use these and other markers clinically, we will need a better way to both study and incorporate multiple patient differences into a single clinical decision. The technological advances in genomics should help us simultaneously analyze an individual patient's "chemotherapy sensitivity" make-up, while advances in information technology will allow us to quickly interpret such results. We hope in the future, that we can then combine these and other markers to provide each patient with an individualized treatment plan.

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