Review

Pharmacogenetics of Solid Tumors: Directed Therapy in Breast, Lung, and Colorectal Cancer

A Paper from the 2008 William Beaumont Hospital Symposium on Molecular Pathology

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Genetic variability in drug-metabolizing enzymes and signaling pathways affects chemotherapy-related toxicity and treatment outcome in cancer. In breast and colorectal cancer, polymorphisms in metabolic enzymes involved in tamoxifen and irinotecan therapies has led the U.S. Food and Drug Administration to address genetic factors relevant to patient consideration of treatment with these compounds. Tamoxifen therapeutic failure in breast cancer has been associated with reduced CYP2D6 activity due to inefficient activation of tamoxifen. Irinotecan toxicity in colorectal cancer is more common in patients with reduced-activity *UGT1A1* alleles, resulting in excessive exposure to the potent SN-38 metabolite. In colorectal and lung cancers, somatic mutations in the epidermal growth factor receptor and downstream signaling molecules have been associated with the therapeutic outcome of epidermal growth factor receptor-directed therapies. This review discusses the current knowledge regarding the utility of single gene—*UGT1A1*, *CYP2D6*, *EGFR*, and *KRAS*—or multigene analysis, for optimizing breast, colorectal, and lung cancer therapy. Current advances in these areas highlight how pharmacogenetics help personalized decision-making for patient management. (*J Mol Diagn* 2009, 11:381–389; DOI: 10.2353/jmoldx.2009.090003)

The goal of personalized medicine is to optimize treatment for individual patients. In oncology, this requires determining the preferred therapeutic agents for each tumor, as well as ascertaining which patients are most

likely to benefit from a given therapy. Individualized cancer treatment has advanced notably in recent years, due largely to growing understanding of the importance of inherited and somatic genetic variability in relation to therapeutic outcome. This review discusses current uses of pharmacogenetics in the management of solid tumors, including optimization of tamoxifen therapy in breast cancer, reduction of irinotecan toxicity in colorectal cancer, and selection of patients for kinase-directed therapies in lung and colorectal cancers. There are other important and growing areas of study pertaining to optimization of oncology therapy through genetic analysis that will not be discussed herein; however, readers are referred to several excellent reviews discussing *TPMT* mutations and thiopurine therapy,¹ *KIT* mutations in gastrointestinal stromal tumors treated with imatinib,^{2,3} and the pharmacogenetics of hematological malignancies.^{4,5}

Tamoxifen Therapy in Breast Cancer

Tamoxifen is approved by the U. S. Food and Drug Administration (FDA) for the treatment and prevention of breast cancer.^{6,7} Administration of tamoxifen in patients with estrogen receptor (ER)-positive breast cancer as adjuvant therapy for 5 years following anthracycline-based chemotherapy reduces the recurrence rate by nearly 50% and the mortality rate by a third after 15 years of follow-up.⁸ The most common side effect of tamoxifen therapy is hot flashes, whereas the most severe adverse effects are the reported incidence of

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This article is partly based on material presented by the authors at the William Beaumont Hospital 17th Annual Symposium on Molecular Pathology: Clinical Applications of Genomic Medicine, which took place on September 10 to 12, 2008, in Troy, MI.

Accepted for publication May 7, 2009.

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Figure 1. Tamoxifen metabolism. CYP3A4/5 and CYP2D6 are the main enzymes involved in tamoxifen metabolism. CYP3A4/5 produces *N*-desmethyl-tamoxifen, the major metabolite, whereas several enzymes including CYP2D6 generate the minor metabolite 4-hydroxy-tamoxifen. *N*-desmethyl-tamoxifen and 4-hydroxy-tamoxifen are further transformed to the active metabolite endoxifen. In patients with CYP2D6 null alleles or potent inhibition, the metabolism of tamoxifen to 4-hydroxy-tamoxifen or endoxifen is blocked.

endometrial cancer and thromboembolic events in tamoxifen-treated patients.⁶

Tamoxifen is considered a prodrug requiring extensive metabolism by cytochrome P450 (CYP) enzymes to elicit its anti-tumor effects, which occur as a result of disrupting the action of estrogen on its receptor.⁹ There are at least three tamoxifen metabolites of clinical interest (Figure 1).^{10–14} The major metabolite, *N*-desmethyl-tamoxifen is roughly equipotent to the parent drug and is produced by CYP3A4/5-mediated demethylation of tamoxifen.¹⁴ In contrast, 4-hydroxy-tamoxifen is generated in small quantities (<10%) by several CYP enzymes including CYP2D6,¹⁵ but has 100-fold greater affinity for the ER and is 30- to 100- fold more potent than tamoxifen in suppressing estrogen-dependent cell proliferation.^{16–18} The third tamoxifen metabolite, endoxifen (4-hydroxy-*N*-desmethyl-tamoxifen) is as potent as 4-hydroxy-tamoxifen in inhibiting estrogen-mediated cell proliferation, but present at concentrations 6- to 10-fold higher than 4-hydroxy-tamoxifen, thus endoxifen is believed to be responsible for the majority of tamoxifen-associated anti-tumor effects.^{13,19,20} Endoxifen is primarily generated by the action of CYP2D6 on *N*-desmethyl-tamoxifen¹⁴; since endoxifen levels depend on CYP2D6 activity, genetic variants of CYP2D6 and drugs that modulate this enzyme could significantly affect outcome in tamoxifen-treated patients.

CYP2D6 and Tamoxifen Therapy

At least 70 CYP2D6 allelic variants exist and are categorized by enzymatic activity into poor, intermediate, extensive, and ultrarapid metabolizers (for an explanation of the categorization of metabolizer status with alleles, see: <http://www.cypalleles.ki.se/cyp2d6.htm>). The frequency of

these variants differs greatly between populations.²¹ For example, CYP2D6*4, one of the most important alleles in conferring a poor metabolizer phenotype, accounts for 12% to 21% of Northern European populations, but only 1% to 2% of Asians and Black Africans.²¹ In contrast, CYP2D6*10 is the most common polymorphism in Asian populations.²² The major CYP2D6 alleles include: wild-type CYP2D6*1, which encodes for a fully functional enzyme; null alleles with essentially no residual activity (CYP2D6*3–*8, *11–*16, *18–*20, *38, *40, *42, *44); reduced-function alleles (CYP2D6*9, *10, *17, *29, *36, *37, *41); and amplified alleles comprised of multiple copies of the CYP2D6 gene (*1XN, *2XN, *35XN, and *41XN).

The influence of CYP2D6 genotype in tamoxifen metabolism has been shown in a number of studies. During tamoxifen treatment, women with two or more fully functional copies of CYP2D6 have higher plasma endoxifen concentrations than patients with one or more nonfunctional alleles (CYP2D6*3 to *6) or those taking known CYP2D6 inhibitors,²³ indicating that reduced CYP2D6 activity results in impaired metabolism of tamoxifen. Analysis of CYP2D6 genotype and endoxifen plasma concentration in 158 patients from multiple ethnicities demonstrated that patients with intermediate metabolizer phenotypes, eg, CYP2D6*10 (reduced activity) or *4 (no activity) heterozygotes, had endoxifen concentrations similar to poor metabolizer phenotypes. In addition, ultrarapid metabolizer patients had higher endoxifen levels than extensive metabolizers, supporting the utility of genotyping CYP2D6 to predict endoxifen concentrations in patients undergoing tamoxifen therapy.

The role of CYP2D6 genetic variants in the treatment outcome of patient taking tamoxifen has also been well documented. A retrospective study of 223 postmenopausal women examined CYP2D6*4, the most common null allele associated with poor metabolizer status.²⁴ Women homozygous for CYP2D6*4 had poorer outcomes than women with CYP2D6*4/*1 or CYP2D6*1/*1 genotypes, showing shorter time to relapse and worse disease-free survival. In addition, no CYP2D6*4/*4 patients experienced moderate or severe hot flashes, in contrast to 20% incidence of this side effect in women with either one or no *4 alleles. Borges et al confirmed these results and expanded the analysis to include 33 different CYP2D6 variants.²⁵ Patients with reduced-activity CYP2D6 alleles (*4, *5, *10, *41) had significantly poorer outcome than carriers of functional alleles, as documented by higher recurrence rates, shorter times to relapse, and worse event-free survival.

Hot flashes, one of the most common side effects of tamoxifen treatment, are commonly treated with antidepressants such as the selective serotonin reuptake inhibitors.⁶ However, some antidepressants are known to inhibit CYP2D6 activity. Co-administration of paroxetine or fluoxetine, both potent CYP2D6 inhibitors, during tamoxifen therapy results in a significant reduction of endoxifen levels in extensive metabolizer patients, as compared with extensive metabolizer patients not using CYP2D6 inhibitors.^{23,25} In contrast, co-administration of venlafaxine, a weak inhibitor of CYP2D6, did not cause significant decreases in endoxifen.²³ Similarly, a recent study as-

sessed the influence of genetic and drug-induced reductions in CYP2D6 activity on breast cancer outcomes in 226 patients co-administered potent or weak/moderate CYP2D6 inhibitors with tamoxifen.²⁶ Patients were stratified by genotype and medication history into subgroups with extensive (normal) or decreased CYP2D6 metabolism. Compared with patients with extensive CYP2D6 metabolism, patients with decreased metabolism showed significantly shorter time to recurrence and worse relapse-free survival. These studies indicate that potent CYP2D6 inhibitors are contraindicated in tamoxifen-treated patients, as the resulting failure to efficiently form endoxifen might jeopardize therapeutic success. Venlafaxine remains a viable option for alleviating tamoxifen-related side effects, as it does not significantly disrupt endoxifen production but is effective in treating hot flashes in breast cancer patients.^{23,27}

Altogether, the current evidence strongly suggests that knowledge of the CYP2D6 genotype may be beneficial when selecting a breast cancer treatment. As such, an FDA advisory panel suggested that the tamoxifen package insert should alert physicians of the following concerns: first, that CYP2D6 poor metabolizer patients are at increased risk for recurrence of their breast cancers if treated with tamoxifen, and second, that co-administration of certain selective serotonin reuptake inhibitors known to inhibit CYP2D6 can affect the metabolism of tamoxifen.²⁸ The limited information that is available on current clinical practice indicates that physicians are acting on these recommendations.²⁹ Knowledge of patient genotype can guide selection of alternate therapies: for example, in postmenopausal CYP2D6 poor metabolizer patients diagnosed with breast cancer, aromatase inhibitors are a reasonable alternative to tamoxifen treatment, or as additional therapy following 2 to 5 years of tamoxifen administration. Randomized clinical trials in postmenopausal breast cancer patients have demonstrated superior efficacy and better overall safety for aromatase inhibitors as compared with tamoxifen.³⁰ For breast cancer prevention, raloxifene appears to be a viable alternative for CYP2D6 poor metabolizer; raloxifene and tamoxifen have been shown to be equally effective in reducing breast cancer incidence in high-risk postmenopausal women.^{31,32}

In the last 5 to 10 years, a great amount of progress has been made in the understanding of how the genetic make-up of patients will influence the response to breast cancer treatment. Unfortunately, most studies have been conducted in postmenopausal women, thus there are few data in premenopausal women to address the relationship between CYP2D6 genotype and the success of tamoxifen treatment, despite the vital role of this compound as the primary anti-hormonal therapy for premenopausal breast cancer patients.

Prediction of Breast Cancer Therapy Success Based on Multigene Analysis

Recently, new approaches such as multigene analysis are being investigated to predict response to breast can-

cer therapy. Unlike CYP2D6 genotyping, which assesses likelihood of successful tamoxifen treatment based on an individual's germline (ie, inherited) genetic makeup, multigene analysis identifies genetic variations occurring specifically within malignant tumor cells to predict response to tamoxifen. The utility of two multigene assays, OncoType DX (Genomic Health, Inc., Redwood City, CA) and MammaPrint (Agendia, Huntington Beach, CA), to guide therapeutic selection for breast cancer is being investigated. Both assays analyze genes involved in cell proliferation, as well as ER and HER-2 signaling pathways. These assays require extraction of RNA from a tumor specimen to assess its gene expression profile, that is, the extent to which each gene is actively playing a role in that particular tumor. This is in contrast to CYP2D6 genotyping, in which DNA is extracted from blood to determine which CYP2D6 alleles a given patient was born with.

The OncoType DX assay is a 21-gene multiplex reverse transcription PCR performed on formalin-fixed paraffin embedded tissue.³³ Based on the expression profile of these 21 genes, OncoType DX determines the 10-year risk for disease recurrence in patients with ER-positive, lymph node-negative tumors using a continuous variable algorithm and assigning a tripartite recurrence score (RS; ≤ 17 , low risk; 18 to 30, intermediate risk; >30 , high risk). A clinical trial, Trial Assigning Individualized Options for Treatment, sponsored by the National Cancer Institute is currently underway to determine whether ER-positive patients with an intermediate RS benefit from chemotherapy or not.³⁴ Patients with an OncoType DX RS ≤ 10 will receive hormonal therapy alone, patients with an RS ≥ 26 will receive hormonal therapy and chemotherapy, and patients with an RS of 11 to 25 will be randomized into either hormonal therapy alone or hormonal therapy plus chemotherapy. Results of the trial are expected to be known by 2013. An initial study demonstrated that OncoType DX was useful in predicting the benefit from tamoxifen in patients with a low or intermediate, but not high RS, and in predicting benefit from chemotherapy in those with a high RS.³⁵ However, the patients used in the analysis were the same patients from which the RS was developed and the thresholds for the three RS groups were defined. Since the RS is dependent on the expression of genes related to ER, HER-2, and proliferation, it is reasonable to predict that it will be informative of the sensitivity to endocrine therapy versus chemotherapy, in general.

The MammaPrint assay uses microarray-based technology to determine the expression profile of 70 genes; it is FDA-approved to assess the likelihood of disease metastasis in breast cancer patients by categorizing them into low or high risk for breast cancer recurrence.^{36,37} A recent study conducted in 198 patients used MammaPrint along with Affymetrix's U133A gene chips to predict disease prognosis, as well as endocrine therapy and chemotherapy sensitivity.³⁸ MammaPrint results categorized 32% of patients as being at low risk for breast cancer recurrence. In these low-risk patients, 31% and 12% were predicted to be highly sensitive to hormonal therapy and chemotherapy, respectively. In the patients categorized as having high risk of recurrence, 51% were

predicted to be chemotherapy-sensitive, 6% were predicted to respond to hormonal therapy, and 42% were predicted to be insensitive to existing therapies. This is the first study to report on the potential utility of the MammaPrint to forecast response to therapy. Additional prospective studies are needed to determine patient outcome, eg, disease-free survival, when treatment is selected according to the therapeutic sensitivity predicted by the tumor gene expression profile.

The impact of multigene analysis in the selection of hormonal and/or chemotherapy is an emerging area. However, it will be a number of years before enough data are available to prove or disprove recent publications in this area. Nevertheless, it is clear that enormous progress is being made in the area of breast cancer treatment to help physicians make personalized decisions related to patient management. The availability of multigene panels and CYP2D6 genotyping puts breast cancer at the forefront of current efforts to tailor oncology therapies for individual patients, and sets the standard for similar endeavors in other areas of cancer treatment.

Irinotecan Therapy in Colorectal Cancer

Irinotecan (Camptosar, CPT-11) is FDA-approved for first-line combined or second-line single-agent treatment of metastatic colorectal cancer,³⁹ and is in clinical trials for a variety of other solid tumors.^{40–42} In combination therapy, such as the FOLFIRI regimen with 5'-fluorouracil and leucovorin, irinotecan is typically given either at three week intervals in high doses, or weekly at lower doses. As a single, second-line agent (usually for 5'-fluorouracil-refractory tumors), irinotecan is given either every week or every other week. Dose recommendations across various irinotecan regimens range from 50 to 350 mg/m² body surface area.³⁹

Irinotecan is a topoisomerase I inhibitor that is both an active cytotoxic agent and a prodrug for its more potent metabolite, SN-38. Most of the absorbed irinotecan is converted by serum carboxylesterases to the major active metabolite SN-38, which is further metabolized by uridine diphosphate-glucuronyltransferase 1A1 (UGT1A1) to inactive SN-38-glucuronide (SN-38-G). Both irinotecan and SN-38 can also be transformed by several CYPs including CYP3A4 to minor, inactive metabolites⁴³; thus, enzyme inhibition from irinotecan therapy may be a concern for patients co-administered other CYP3A4 substrates. Toxicity is a major concern of irinotecan therapy, and can be either immediate or delayed.³⁹ Early adverse responses include diarrhea and other cholinergic symptoms, while late-onset toxicity occurs at least 1 day after irinotecan administration, and is characterized by potentially life-threatening diarrhea, neutropenia, or both.⁴⁴ In general, the appearance of severe, delayed adverse responses correlates to SN-38 exposure.⁴⁵

The primary metabolic pathway for irinotecan is conversion to SN-38, with glucuronidation to the more readily excreted SN-38-G.⁴⁶ UGT1A1, which is heavily implicated in the formation of SN-38-G, is actually one of at least ten related proteins encoded by the UGT1A gene

Table 1. Clinically Relevant UGT1A1 Polymorphisms

Allele	Polymorphism/Mutation type, location	Residual activity
<i>UGT1A1</i>		
*1	Wild-type	100%
*28	7 TA repeats, promoter	20% to 70%
*36	5 TA repeats, promoter	Uncertain, increased activity >*1?
*37	8 TA repeats, promoter	Uncertain, <50%?
*6	SNP, exon 1	30% to 50%
*27	SNP, exon 1	30% to 50%
*7	SNP, exon 1	10% to 20%

locus.^{47,48} Of these, UGT1A1 has received the most attention, since deficiency of this enzyme is responsible for hereditary diseases (Gilbert and Crigler-Najjar syndromes). Reduced expression or activity of UGT1A1 also prolongs exposure to SN-38, increasing risk of toxicity.⁴⁹

Of the germline variants characterized to date, the most common allele resulting in reduced UGT1A1 activity is a promoter polymorphism with 7 TA (thymine-adenine) repeats, which reduces transcriptional activity relative to the more common six-repeat allele (Table 1).⁴⁷ The seven-repeat allele (UGT1A1*28) has a homozygous frequency as high as 10% to 20% in some ethnic groups. Variants have also been described with five (*36) and eight (*37) repeats; these appear to be more common in African-Americans.⁵⁰ Though the rarity of these latter two alleles makes thorough study difficult, UGT1A1 activity seems to be inversely correlated with the number of TA repeats: the five-repeat allele shows equal or greater activity compared with the six-repeat allele, while the eight-repeat variant results in worse deficiency than the seven-repeat allele.⁵¹ Irinotecan toxicity has been associated with reduced UGT1A1 activity, thus both *28 and *37 alleles would be predicted to increase risk of adverse events.

Initial studies linking the homozygous seven-repeat (*28/*28) genotype to severe, late-onset irinotecan toxicity led to modification of the package insert, suggesting reduced dosage for these patients.^{44,52} Identification of the *28/*28 genotype was facilitated by FDA approval of a rapid testing kit (Invader UGT1A1), and by development of in-house methods elsewhere. Despite the focus on the seven-repeat allele, it is well-known that other UGT1A1 alleles can result in reduced enzyme activity and thereby increase SN-38 exposure.⁵³ These variants are not addressed by the irinotecan package insert or the FDA-approved assay, despite evidence of impaired SN-38 glucuronidation and thus a predicted link with irinotecan toxicity.

Several of the non-promoter UGT1A1 variants are missense mutations found predominantly in Asians. The most common is UGT1A1*6 (allele frequency ~20% in Asians), which results in alteration of arginine 71 to glutamine.⁵⁴ UGT1A1*6 has roughly half the *in vitro* SN-38 glucuronidating activity of the UGT1A1*1, and has been implicated in irinotecan toxicity in Asians.^{55,56} A less-common allele (frequency ~3% of Asians), UGT1A1*27, is a Pro229Gln variant with comparable activity to UGT1A1*6. Finally,

UGT1A1*7 is a very rare variant resulting in a Tyr486Asp change, which severely reduces enzymatic function and produces a marked hyperbilirubinemia in the homozygous state⁵⁵; heterozygotes would be predicted to be at increased risk of irinotecan toxicity.

However, it is not yet clear whether hereditary reductions in UGT1A1 activity always predispose a patient to irinotecan toxicity. The predictive capacity of the *28/*28 genotype is poor: risk of adverse effects is increased, but far from certain. It may be that the degree of association between the *28 allele and severe toxicity depends greatly on the specific dosing schedule studied.⁵⁷ High-dose (>250 mg/m², every 3 weeks) regimens are more likely to show increased risk of toxicity in *28/*28 patients than are low-dose (<150 mg/m², weekly) schedules.⁵⁷ This agrees with pediatric studies of low-dose irinotecan, which show little association between the *28 allele and toxicity.^{58,59}

Thus, the influence of UGT1A1 genotype might be mitigated by treating susceptible patients with low-dose regimens, while recommendations for dose adjustment might require protocol-specific tailoring. Moderate-dose irinotecan can be successfully combined with other agents,⁶⁰ yet is only associated with minor increases in likelihood of toxicity for *28 patients.⁵⁷ Thus, individuals who are willing to tolerate some risk could potentially receive the benefit of full-dose, combined chemotherapy regardless of UGT1A1 genotype. In contrast, patients with wild-type UGT1A1 may well be underdosed, since dosing protocols were established without genetic stratification. Excretion of glucuronidated chemotherapeutics has been associated with drug resistance in cancer cells,⁶¹ thus insufficient exposure to SN-38 may be a concern in patients with adequate UGT activity. However, these speculations require empirical confirmation; prospective studies of irinotecan dosing by UGT1A1 activity are sorely lacking, though genotype-specific dose-escalation trials may soon provide much-needed data in this regard.⁶²

Several other UGT enzymes can glucuronidate SN-38 *in vitro*, including 1A7, 1A9, and 1A10; polymorphisms in any of these could theoretically affect irinotecan pharmacokinetics.⁴⁷ It appears that UGT1A9 variants are rare, at least in Europeans, and do not significantly affect irinotecan metabolism *in vivo*.⁶³ In contrast, UGT1A7 polymorphisms are fairly common and often show reduced SN-38 glucuronidation.⁴⁷ Studies to date are limited and somewhat conflicting: several show no effect of UGT1A7 variants,⁶⁴ while one linked reduced-activity UGT1A7 alleles to enhanced anti-tumor response and lower incidence of diarrhea.⁶⁵ A recent, comparatively large study correlated UGT1A7 variants with diarrhea, thrombocytopenia, and anemia after irinotecan combination therapy.⁶⁶ There was no link between UGT1A1*28 and toxicity; however, known Gilbert's patients were specifically excluded, suggesting that the study group might display milder UGT1A1 phenotypes than would a non-selected population of *28 carriers.

Analysis of the entire UGT1A gene may be more informative than studies of single isoforms. The inconsistencies between reports may reflect different haplotypes of variant

UGT alleles: low-activity UGT1A7 and UGT1A9 polymorphisms are in linkage disequilibrium with UGT1A1*6,⁶⁷ while UGT1A7 variants are often found in UGT1A1*28 carriers.⁶⁸ Thus studies identifying only one polymorphism may be confounded by combinations of variant UGT1 isoforms. Much work remains to clarify the relative importance of UGT enzymes in SN-38 glucuronidation; future studies would ideally incorporate a wide range of ethnic groups to address unequally distributed alleles. Prospective genotyping of the UGT1A locus will undoubtedly facilitate study of irinotecan pharmacokinetics and toxicity, and hopefully further the goal of optimizing chemotherapy for individual patients.

Epidermal Growth Factor Receptor-Directed Therapy in Colorectal and Lung Cancer

Another recent development in colorectal cancer therapy is the association between somatic tumor-specific mutations in pathways related to epidermal growth factor receptor (EGFR) signaling and the therapeutic efficacy of EGFR inhibitors. EGFR is one of a family of tyrosine kinase receptors that also includes HER2/neu; the success of the anti-HER2/neu directed therapy trastuzumab (Herceptin) in breast cancer was followed rapidly by an influx of medications targeting EGFR, including monoclonal antibodies (cetuximab, panitumumab), specific kinase inhibitors (gefitinib, erlotinib), and broader-spectrum kinase inhibitors (lapatinib, canertinib).^{69–71} These targeted therapies are expected to have the greatest benefit in tumors with strong dependence on EGFR-mediated signaling, such as those with EGFR gene amplification or gain-of-function mutations in EGFR.⁷²

EGFR is a membrane receptor with an intracellular kinase domain; on ligand binding and dimerization, EGFR stimulates at least two potent signaling cascades affecting cell outcome.⁷¹ The dominant pathways are the mitogen activated protein kinase (MAPK) and phosphoinositol-3 kinase (PI3K) networks (Figure 2), which regulate transcription of genes involved in survival, proliferation, invasion, and metastasis. Several genes encoding key components of these cascades have been implicated in the success of EGFR-directed therapy, including Raf, which stimulates MAPK; Akt, which enhances signaling downstream of PI3K; and Ras, which directly activates both MAPK and PI3K pathways.^{73,74} Of these, the *KRAS* gene has been most extensively characterized for its role in chemotherapy outcome.

Gain of function mutations of *KRAS* occur most frequently in codons 12 and 13, though there are other less-common alterations described that result in a constitutively active protein.⁷¹ Activating mutations in *KRAS* are generally mutually exclusive from those of EGFR: both gene products stimulate MAPK and PI3K signaling, thus there is no selective pressure for tumors to activate sequential steps in the same cascade.⁷¹ However, in the context of EGFR-directed therapy, alterations in these two genes have very different consequences. Most activating mutations of EGFR enhance tumor susceptibility to therapeutic agents (inhibitors or antibodies) directed

Figure 2. EGFR-dependent signaling. Dimerization of EGFR stimulates phosphorylation (P) of its cytoplasmic domain and enhances signaling (arrows) via several pathways. Ras proteins including K-Ras activate Raf and phosphoinositide-3 kinase to stimulate the MAPK and PI3K pathways, respectively; PI3K can also be activated directly by EGFR. MAPK and PI3K signaling affects transcription of genes involved in regulating proliferation, apoptosis, and other cellular processes. EGFR-directed therapies such as monoclonal antibodies and kinase inhibitors inhibit (bars) discrete steps in these signaling networks.

against the receptor, while KRAS gain-of-function bypasses EGFR entirely and therefore results in extremely poor response to directed therapies.⁷⁴

EGFR-directed agents are approved for use in lung and colorectal cancers. Studies in these patients have confirmed that mutations in EGFR or KRAS can affect therapeutic outcome. Nonetheless, tumors with amplified or activated EGFR tend to show better response to targeted therapies such as gefitinib and erlotinib.⁷⁰ Most small deletions or point mutations in the kinase region increase susceptibility to specific EGFR inhibitors, although some somatic mutations (eg, Tyr790Met) have been found to confer resistance and can occur as secondary alterations after initially successful EGFR-directed therapy.⁷⁴ EGFR amplification can co-exist with activating mutations in the gene; in these cases the mutant allele is typically the one amplified, suggesting that selective pressure improves survival of the tumor cells with multiple copies of the constitutively active receptor.⁷⁴ Interestingly, EGFR mutations in lung cancer are more common in East Asian populations, including subsets with relatively favorable prognoses such as Asian female never-smokers.⁷⁰

Notably, most studies show that a subset of patients with apparently normal EGFR expression nonetheless respond well to EGFR-directed therapy, suggesting that there may be additional mutations not currently detected by common study protocols, or that some tumors may depend sufficiently on EGFR signaling to be susceptible to therapeutics targeting that pathway.⁷⁰ It must be noted that most studies assess only a limited number of mutation “hot spots” in these two genes, potentially confound-

ing interpretation of results. In addition, most studies have addressed somatic mutations, but heritable polymorphisms in EGFR and related genes may also be important: one germline polymorphism results in an Arg521Leu amino acid change in the EGFR extracellular domain, and correlates with improved outcome after cetuximab treatment.⁷⁵ Future studies including full-gene sequencing rather than assessment of select regions may clarify this situation.

In contrast to activating EGFR mutations, which correlate with successful response to directed therapy, lung tumors with somatic alterations in KRAS show negligible response (<5% of patients) to EGFR-targeted agents.^{71,76–78} Of note, many tumors with normal KRAS also fail to respond to EGFR-directed therapy, thus wild-type KRAS is a poor predictor of outcome, but the presence of activating mutations essentially guarantees that alternate treatment modalities will be required. The situation is much the same in colorectal cancer, where the incidence of KRAS mutations is higher (36% compared with 20% in lung cancer).⁷¹ The effect of KRAS mutations on EGFR-directed therapeutic failure has been seen with both antibody^{79–81} and small molecule inhibitor⁷⁶ agents. Overall and progression-free survival rates are typically greater for tumors without KRAS mutations; it is likely that the published differences—though already remarkable—would be even more statistically significant with more sophisticated stratification of patients according to known risk factors in addition to KRAS status.

However, the paucity of large, prospective trials assessing the role of KRAS or EGFR mutations has not deterred clinicians from modifying current practice to reflect the available data. The European Medicines Agency recently altered approval for cetuximab to include combined or single-agent therapy in EGFR-expressing KRAS-wild-type colorectal tumors⁸²; this decision is supported by recent results indicating improved survival in KRAS-wild-type tumors treated with this agent.⁸³ Likewise, approval for panitumumab was limited to use in KRAS-wild-type tumors,⁸⁴ sparking expanded distribution of a commercial test for KRAS mutations in colorectal cancer. Though the FDA has yet to issue recommendations similar to these, individual investigators have suggested that prospective genotyping for EGFR and KRAS should become standard practice for management of lung and colorectal cancer patients. Given the comparatively mild side effects associated with EGFR-directed therapies, optimizing their use in cancer management provides hope for effective therapy without the devastating impact to quality of life seen with traditional chemotherapies.

Summary

The advent of pharmacogenetic applications for oncology has begun the long-awaited process of creating tailored therapeutic plans for individual patients, but there is much work remaining. Study of hereditary polymorphisms in metabolic enzymes has allowed clinicians to identify subsets of patients likely to respond favorably

or poorly to tamoxifen and irinotecan. However, the significant variability in drug-metabolizing enzyme genes suggests that these findings are only the beginning; further refinement of patient selection requires greater understanding of the primary and secondary metabolic pathways for these compounds, as well as clarification of the importance of individual polymorphisms. Likewise, a handful of somatic mutations affecting genes in the EGFR pathway show great promise for optimizing patient selection for EGFR-directed therapies, but the incredible complexity of this signaling network implies that there are many other potential markers for response yet to be understood.

Much work remains for those seeking to improve pharmacogenetic applications in chemotherapy. One glaring need common to breast, colorectal, and lung cancers is the paucity of large, prospective studies incorporating comprehensive molecular testing. The low incidence of certain germline polymorphisms complicates analysis of UGT1A, CYP2D6, and other metabolic enzymes implicated in irinotecan and tamoxifen treatment, but there is evidence that less-frequent alleles may play vital roles in therapeutic outcome. As well, little attention has been given to potential transcriptional regulation through elements in the 3'-untranslated regions of these genes. Thus characterization of somatic mutation hot spots can speed and simplify data interpretation, but may overlook mutations of therapeutic significance. Comprehensive studies are daunting and expensive, but may be essential to clarifying the complex, multifactorial role of genetics in determining patient response. Regulatory recommendations are a commendable first step in increasing recognition and clinical application of genetic information in oncology, but further refinement of these guidelines will undoubtedly be required as understanding of the pharmacogenetics of cancer therapy improves.

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