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Pharmacogenomics

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DEFINITIONS AND OBJECTIVES

Genomics is defined as the comprehensive study of the whole set of genes, gene products and their interactions (1). Pharmacogenomics (PG) can be considered as the study of pharmacologically relevant genes, the way they manifest their variations, how these variations interact to produce "phenotypes", and how these phenotypes affect drug response (2). By increasing ability to identify patients with risk for severe toxicity, or those likely to benefit from a particular treatment, PG is surely leading towards individualized cancer therapy (3). The aim of personalized medicine or individualized treatment is to match the right drug to the right patient.

Significant heterogeneity in the efficacy and toxicity of chemotherapeutic agents was observed. The goal of PG is to check the genetic background of a patient in order to ensure that the prescribed drugs are effective and free from side effects. The ultimate goal of cancer PG is to develop diagnostic test predictive of therapeutic response, identifying patients who will respond well to specific treatment and those with high risk for severe drug induced toxicity (Table 1).

Table 1. Objectives of cancer pharmacogenomics

To identify patients at increased/decreased probability of toxicity/benefit from drug
To identify tumors at increased/decreased probability of antineoplastic effect from drugs
To measure effect of drugs on normal/malignant tissue

Identification of single nucleotide polymorphisms (SNPs) and haplotypes in normal cells (peripheral blood, buccal smears, skin) can be used to predict drug toxicity, while diagnostic tests predictive of efficacy will be based on markers in tumor cells. Currently we are using three most important analytical tools in PG, genotyping, loss of heterozygosity, and mRNA expression analysis, to compare genetic markers in tumors from responsive and non-responsive patients.

Table 2 and the following text represent several clinically relevant examples of gene polymorphisms that alter both the toxicity and the efficacy of some anticancer drugs.

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Table 2. Examples of polymorphisms associated with variable drug responses

Protein	Drug	Polymorphism and consequence
Thiopurine methyltransferase	6-Mercaptopurine (6MP)	Toxicity and efficacy of 6MP in leukemia (ALL)
Dihydropyrimidine dehydrogenase	5-FU	5-FU toxicity due to 5-splice recognition-site mut.
UGT1A1	Irinotecan	Metabolism and toxicity of Irinotecan
ERCC1, XPD and GST	Platinum analogs	Polymorphism decrease response and survival
SULT1A1	Tamoxifen	Influence on results in adjuvant therapy
MTHFR	Methotrexate	Point mutation, TT patients have increased risk of toxicity

THIOPURINES (MERCAPTOPURINE, THIOGUANINE, AND AZATHIOPRINE)

Thiopurines are inactive agents that require activation to thioguanin nucleotides (TGN) to exert cytotoxicity. This activation is catalyzed by multiple enzymes, and first of them is hypoxanthine phosphoribosyl transferase (HPRT). On the other side, these drugs can be inactivated via oxidation by xanthine oxidase (XO) or via methylation by thiopurine methyltransferase (TPMT). By S-methylation, TPMT is shunting these drugs away from TGN formation. TPMT polymorphisms are associated with the therapeutic efficacy and toxicity of mercaptopurines. Approximately, 90% of the human population has high TPMT activity, about 10% have intermediate activity and 0.3% have low or no detectable activity. Studies have shown that TPMT-deficient patients are at very high risk of developing severe and life threatening myelosuppression if treated with conventional doses of thiopurines (3,4). Also, patients who are heterozygous at the TPMT locus are at intermediate risk of dose-limiting toxicity (5,6).

5-FLUOROURACIL (5-FU AND ORAL PRODRUG CAPECITABINE)

5-FU is an excellent example of the way in which genetic variation a drug-metabolizing enzyme (DPD) and a drug target (TS) can influence both toxicity and response to the treatment. 5-FU is a prodrug that requires activation to 5-fluoro-2-deoxyuridine monophosphate (5-FdUMP). 5-FdUMP inhibits tumor cell replication via inhibition of thymidylate synthase (TS), an enzyme that is required for de novo pyrimidine synthesis (Figure 1).

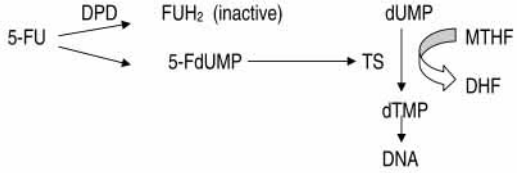

Figure 1. Activation and inactivation of 5-fluorouracil

In humans, up to 85% of an administered intravenous dose of 5-FU is degraded in the liver by dihydropyrimidine dehydrogenase (DPD), an enzyme that exhibits up to 20-fold variation in activity among individuals. Patients with low DPD activity cannot effectively inactivate 5-FU, leading to excessive amounts of 5-FdUMP, causing potential fatal gastrointestinal, hematopoietic and neurological toxicities (peripheral neuropathy, encephalopathy and demyelination) (7). The most common inactivating allele of DPD is caused by a G—›A alteration at the invariant GT splice donor site flanking exon 14. This allele (DPYD*2A) causes the skipping of exon 14, and leads to production of a non-functional protein, which is associated with severe toxicity and fatal outcomes of 5-FU treatment in some studies.

Genetic polymorphisms in the gene encoding thymidylate synthase (TS) have also been shown to influence response to 5-FU therapy. Many studies indicated that both TS mRNA and protein levels are inversely related to clinical antitumor response; survival of patients with advanced colorectal cancer is inferior if TS expression is high (8).

IRINOTECAN

Irinotecan, a topoisomerase I inhibitor itself is a prodrug, which requires activation by carboxylesterase (CE) to its active metabolite, SN-38. Hepatic UDP-glucuronosyl-transferase 1A1 (UGT1A1) glucuronidates SN-38 to form more polar and inactive SN-38 glucuronide, which is eliminated in the bile and urine. Both gastrointestinal (diarrhea) and hematologic (neutropenia) toxic effects are dose limiting after administration of irinotecan, and they are associated with increased levels of SN-38. The clinical pharmacogenetics of irinotecan treatment is focused on polymorphic glucuronidation of SN-38 by UGT1A1. UGT1A1 expression is highly variable in the rate of up to 50-fold (9). Because of the clinical importance of the glucuronidation pathway in irinotecan treatment, UGT1A1 was chosen as the candidate gene to be investigated as a predictor of severe toxicity.

PLATINUM ANALOGS (CISPLATIN, CARBOPLATIN AND OXALIPLATIN)

These agents inhibit cellular replication by forming inter and intrastrand helix-deforming DNA adducts (10). Resistance to platinum agents can occur because of decreased drug accumulation, detoxification through conjugation, enhanced tolerance to platinum-induced DNA adducts or enhanced DNA repair (11). The nucleotide excision repair pathway, which is involved in the repair of many DNA lesions, includes several well-defined genes such as excision repair cross-complementation group 1 and xeroderma pigmentosum group D (ERCC1 and XPD) that encode proteins involved in the removal of cisplatin-DNA adducts. Recent studies indicated that genetic polymorphisms in these repair genes as well as genes encoding proteins in other DNA repair pathways, X-ray cross-complementing (XRCC1), may influence response to platinum chemotherapy.

The XPD protein (helicase) takes part in DNA transcription and in the removal of DNA lesions induced by platinum chemotherapy. A nonsynonymous SNP, altering a lysine to glutamine at codon 751 of the XPD protein was shown to be clinically significant. In a retrospective study, in which colorectal cancer patients received oxaliplatin plus 5-FU, those with *XPD Lys751Gln* polymorphism (either homozygous or heterozygous) had significantly decreased response rates and survival compared with homozygous for the *Lys751/Lys751* genotype (12). 24% of patients with the lysine/lysine genotype achieved an objective response to therapy, compared to only 10% of those with the lysine/glutamine or glutamine/glutamine genotypes. Also, patients with the lysine/lysine genotype had a median survival of 17.4 months, compared to 12.8 months for lysine/glutamine heterozygotes and 3.3 months for glutamine/glutamine homozygotes (12).

The XRCC1 gene encoded enzyme involved in the repair of single-strand interruptions in DNA. A polymorphism in the XRCC1 gene, SNP that encodes either an arginine or a glutamine at codon 399 of the protein, was significantly associated with treatment response.

Polymorphisms in glutathione dependent enzymes have also been indicated to influence response to platinum chemotherapy agents. Glutathione-S-transferases (GSTs) catalyze the conjugation of glutathione to a wide variety of toxic compounds including platinum agents, and form less toxic and water-soluble conjugates that are exported out of target cells. There are five subclasses of GST family (GSTA1, GSTP1, GSTM1, GSTT1 and GSTZ1) that influence cytotoxicity to a variety of chemotherapeutic agents (49). Stoehlmacher et al. (13) recently showed that one SNP in GSTP1 was associated with overall survival in 107 patients with metastatic colorectal cancer who received 5-FU/oxaliplatin combined chemotherapy. The result of this SNP is replacement of isoleucine with valine at position 105 of the protein, which leads to diminished enzyme activity. In this study, the valine homozygotes had a median survival of 24.9 months compared to heterozygotes with median survival of 13.3 months, and isoleucine homozygotes with median survival of 7.9 months.

TAMOXIFEN

Tamoxifen is used in the treatment of all stages of hormone-dependent breast cancer as well as in the prevention of breast cancer. First, tamoxifen must be metabolized in 4-hydroxytamoxifen, which is about 100-fold more potent as an antiestrogen than is tamoxifen (14). Thus far, four major sulfotransferases (SULTs) have been discovered in the human liver. Among them, SULT1A1 has the most important role in the hepatic cytosolic trans-selective sulfation of 4-hydroxytamoxifen isomers. A single nucleotide polymorphism in the SULT1A1 gene results in an arginine to histidine substitution at codon 213. Individuals homozygous for the His allele have about a 10-fold lower SULT activity compared with individuals with high-activity allele (SULT1A1*1) (15). In a recent retrospective study it was showed that, among women who received tamoxifen as adjuvant treatment of breast cancer, homozygous for the SULT1A1*2 (low-activity allele) had approximately 3-fold greater risk of death compared to homozygous for the common allele or heterozygous (SULT1A1*1/*2) (16). Among women who did not receive tamoxifen, association between survival and SULT1A1 genotype was not found.

METHOTREXATE

Folate has an important role in methyl group metabolism and its disorders may result in decreased availability of nucleotides for DNA synthesis and alterations in DNA methylation. Folate metabolism depends on two major factors: folate intake and proper activities of enzymes involved in its metabolism. Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme that regulates the metabolism of folate and methionine, both of which are important factors in DNA methylation and synthesis. MTHFR is polymorphic enzyme, which irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate that is linked with production of S-adenosyl-methionine, a universal donor of methyl group. Transfer of methyl group is necessary for remethylation of homocysteine to methionine and conversion of dUMP to dTMP. MTHFR gene is located on chromosome 1p36.3 and is composed of 11 exons. Most common mutation of this gene is C677T which substitutes valine for alanine, resulting in thermolabile enzyme variant with reduced activity. This leads to lower plasma folate level and elevated homocysteine level. Approximately 10% of the population are homozygous for the 677T variant that encodes an enzyme with about 30% of the wild-type enzyme activity, and 40% are heterozygous with 60% of the wild-type enzyme activity. Another MTHFR mutation, A1298C may also decrease its activity when coexisting with the previous one (17).

Methotrexate (MTX) is an antifolate chemotherapeutic drug used in the treatment of lymphoma, solid tumors and also rheumatoid arthritis or very severe forms of psoriasis. Toxicities include mucositis and myelosuppression (neutropenia, thrombocytopenia, anemia). MTX treatment increases serum homocysteine and induces a low folate level. MTX, by affecting the intracellular folate pool, influences the activity of the enzyme MTHFR. Consequently, patients with decreased MTHFR activity are at an increased risk of MTX-related toxicity. For example, compared with patients with wild-type genotype, those with 677TT genotype are at an increased risk of MTX-induced oral mucositis, a complication caused by delayed healing because of decreased synthesis of nucleotides and impaired ability of DNA repair (18). Interestingly, in patients with MTHFR 677CT alleles, increased risk of MTX-induced toxicity was reported only after low-dose MTX (18), and not after high-dose MTX with leucovorin rescue. It is possible that leucovorin rescue attenuates the increased risk of toxicity by providing an exogenous source of reduced folates that compensates low folate levels in these patients. In conclusion, studies suggest that the TT MTHFR 677 genotype is associated with marked MTX-induced hyperhomocysteinemia and could represent a pharmacogenomic marker for toxicity after chronic treatment with low doses of MTX.

MICROARRAYS IN CANCER PHARMACOGENOMICS

Examples just mentioned represent situations in which a small number of gene exert a major effect on drug response. But, for most of anticancer drugs, it is possible that drug response is much more complex, with multiple polymorphic genes and environmental factors contributing to overall treatment outcome (3). Consequently, in order to understand better the genetic basis of drug response, genome-wide researches are needed. Recently, microarray analysis have been applied to the filed of cancer PG. The development of microarrays has revolutionized the way gene expression is evaluated in oncology. To analyze gene expression with microarrays, target nucleic acids after extracting from tissue, have to be labeled with a fluorescent dye. By monitoring the amount of label that has hybridized to each location on the microarray, plenty of multiple transcripts can be measured simultaneously. Recent studies have also shown that transcriptional profiling has great potential for assigning known tumors to groups that can predict outcome or response to therapy (19,20).

CONCLUSION

PG has great potential to revolutionize cancer medicine. Microarray has shown great chances for individualizing cancer therapy in two ways: either through better diagnosis of subgroup with risk, or by direct markers of chemosensitivity. In addition, PG may lead to the more efficient development of novel cancer therapies. Since the PG will become one of the key platforms for personalized medicine, it is important to incorporate its educational aspects into medical school curricula (21).

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