

CONTINUING EDUCATION IN ONCOLOGY

Basics of cancer pharmacogenomics

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Definitions and potential objectives

Significant heterogeneity in the efficacy and toxicity of chemotherapeutic agents is observed across the population. Administration of the same dose of an anti-cancer drug given to a group of cancer patients results in a range of side effects from unaffected to lethal events. Age, gender, organ function, tumor biology, all are associated with drug response, but genetic differences in drug disposition and drug targets can have great impact on treatment results. The metabolic enzymes and cellular

targets for the majority of the chemotherapeutic agents contain genetic polymorphisms. Pharmacogenomics is the study of how genetic inheritance influences response to drugs. It can be also defined as the study of genetic variations causing variable drug response and includes the study of genetic polymorphism of drug transporters, drug metabolizing enzymes and drug receptors. Genomics is defined as the comprehensive study of the whole set of genes, gene products and their interactions [1]. Pharmacogenomics is the research area which aim is to identify disease genes and new drug response markers. Pharmacogenomics 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, pharmacogenomics is surely leading towards individualized cancer therapy [3]. A systematic understanding of the genes that modulate response to drugs may change the way they are prescribed. The goal is to check the genetic background of a patient in order to ensure that the prescribed drugs are effective and free from side effects.

Pharmacogenomics is probably more crucial to the treatment of cancer than to any other illness. Generally, current anticancer therapies have exhibited limited success. Also, the consequence of administering optimal therapy, which employs high doses of extremely toxic drugs, is severe – cancer may still advance while patients suffer potentially life-threatening side effects. Pharmacogenomics can lead to optimized therapeutic regimens, resulting in improved quality of life and increased life expectancy for cancer patients. The ultimate goal of cancer pharmacogenomics is to develop diagnostic tests 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).

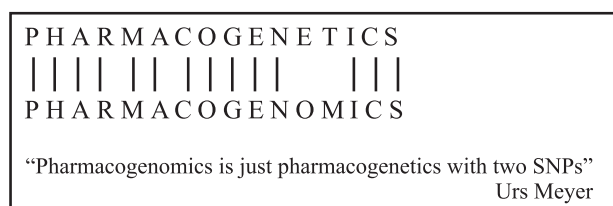
Table 1. Potential objectives of cancer pharmacogenomics studies

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

Such tests are particularly important in oncology because of the variety of anticancer therapies. Diagnostics which guide the choice of an individual patient's therapy, promise to significantly improve the long-term outcome of cancer treatment. The first step in developing molecular diagnostics is to identify genetic markers predictive of efficacy or toxicity. 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 can be based on markers in tumour cells (surgical specimens, hematological malignancies, biopsy). There are three most important analytical tools, genotyping, loss of heterozygosity and mRNA expression analysis, to compare genetic markers in tumors from responsive and non-responsive patients.

Genotype/Haplotype analysis

Most genes contain multiple DNA alterations, including SNPs, which may affect therapeutic response. The first step in applying pharmacogenomics to cancer treatment is to define the genetic variability in genes predicted to affect response to a drug. After screening, a subset of polymorphisms is selected for further analysis using criteria such as allele frequency, linkage disequilibrium with other SNPs and molecular modeling. Haplotyping identifies the groups of polymorphisms that occur in each gene, and reduces by one to three orders of magnitude the number of SNPs combinations. SNP is a stable single base sequence alteration that occurs in more than 1% of the population. It is the simplest type of DNA polymorphism (Figure 1).

**Figure 1.** Single nucleotide polymorphism (SNP).

Among more than 1.4 million SNPs identified in the human genome thus far, more than 60,000 occur in the coding region of genes, and some are associated with variations in drug metabolism and effects [4]. Other types of polymorphism include a variable number of tandem repeats (also known as minisatellites), which consist of multiple copies of repeated DNA sequences (0.1 - 10kb), and microsatellite repeats, a more common simpler variant of minisatellites. SNPs have several advantages over microsatellite repeat markers for fine mapping for several reasons. Firstly, SNPs are much more frequent than microsatellite repeats and occur, on average, once every 500–1,000 bp, which means that there are about 3 to 6 million SNPs spread throughout the human genome [5]. Secondly, SNPs are less prone to germline mutations, which means that their inheritance is more stable. Finally, SNPs are mostly bi-allelic, which makes population frequency estimations easier [6,7]. For these reasons, SNPs have become the marker of choice for association studies in pharmacogenomic applications. The current effort is to identify polymorphisms, especially in gene regulatory or coding regions, that have clinical relevance. Genetic polymorphism is often associated with reduced activity of the encoded protein, but some are associated with enhanced activity. SNPs are generally identified by high throughput sequencing of cloned DNA segments from panels of 10 to 50 ethnically diverse subjects. There is now an increasing number of SNPs that are correlated with severe toxicity of chemotherapeutic drugs.

Loss of heterozygosity

Cancer cells typically lose up to 20% of their genome, resulting in the tumor having only one copy of many vital genes. This phenomenon, known as loss of heterozygosity (LOH), is an important determinant of gene activity in the tumor. When this LOH affects tumor suppressor genes, the result may be a growth advantage due to loss of tumor suppressor function. Cells with accelerated growth then develop into tumors in which each cell carries this LOH pattern. For example, LOH at the p53 and RB loci is associated with poor response to chemotherapy in the treatment of osteosarcomas. In addition to affecting tumor suppressor genes, LOH impacts the expression and activity of thousands of other genes that are present in only one allelic form in the tumour. For example, if a patient possesses high and low activity alleles of a drug target, and if high activity allele is lost as a result of LOH, then activity is lower in the tumour than in normal tissue. If such a gene is targeted by an inhibitory drug, a lower activity target is likely to

be associated with increased responsiveness to the drug. Both the genetic differences among tumors and the normal genetic variations between individuals contribute to inter-patient variation in therapeutic response. There is an increasing body of evidence that the pattern of LOH in a tumor does influence its susceptibility to drug treatment. LOH is typically assessed using microsatellite assays, in which PCR is used to amplify polymorphic microsatellite markers capable of distinguishing maternal and paternal copies of a chromosome. Ultimately, LOH could serve more than just a prognostic marker - it could be exploited to selectively kill cancer cells by targeting differences between tumor and normal tissue [8].

mRNA expression analysis

Expression profiling is widely used to simultaneously measure the mRNA levels of a large number of genes. Expression levels of specific genes have been shown to predict response to chemotherapy with great accuracy. Comparing mRNA expression patterns of responsive and non-responsive tumors we could see differences that may be critical to drug response. Attention must be focused on genes involved in the biological pathway of drug action. The predictive power of such associations has been illustrated in the case of 5-FU. Low intratumoral levels of the mRNAs for thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD) and thymidine phosphorylase (TP) are associated with increased responsiveness to 5-FU. Since the expression levels of these three genes are independent variables, they can be combined to increase the predictive power of transcriptional analysis. Patients with low levels of TS mRNA had about 57% response rate, those with low TS plus low TP had 79% response rate and low TS with low DPD 92% response rate. When expression levels of all three genes were low, 100% of the tumors responded to 5-FU, while high expression of any of these genes, made the tumor unresponsive to treatment [9]. A major challenge for this method is that mRNA is most reliably obtained from fresh, snap frozen tumor samples, which are not often available.

In addition to ensuring better medical treatment, pharmacogenomics may lead to a more efficient development of novel cancer therapies. In the early stages of drug development, pharmacogenomics can guide target selection towards targets with limited genetic variability. During clinical trials, genetic markers associated with clinical response can be used to stratify patients into groups, depending on their predicted response to treatment. This could lead to smaller and more definitive clinical trials. Finally, for drugs that demonstrate high toxicity or low efficacy, pharmacogenomics could lead to safer and more efficacious usage. In short, pharmacogenomics is expected to significantly improve the medical care for cancer patients by ensuring that each individual receives the most efficacious and least toxic anticancer drug.

In this presentation, we will focus on several selected, clinically relevant examples in which variation in genes encoding proteins that influence drug metabolism, drug transport and drug targets can alter both the toxicity and the effectiveness of some commonly used anticancer drugs.

Thiopurines

Thiopurines are a family of drugs that includes mercaptopurine (used for childhood ALL treatment), thioguanine (used to treat AML) and azathioprine (commonly used immunosuppressant in solid organ transplants, rheumatic and dermatologic diseases). The cytotoxic mechanism of these drugs is the incorporation of thioguanine nucleotides into DNA (Figure 2). However, thiopurines are inactive agents that require activation to thioguanine nucleotides (TGN) to exert cytotoxicity. This activation is catalyzed by multiple enzymes, the first of them being hypoxanthine phosphoribosyl transferase (HPRT). On the other hand, 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.

Table 2. Examples of polymorphisms associated with variable drug responses

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

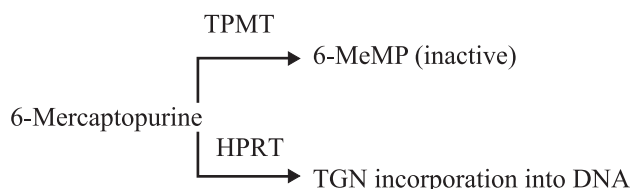


Figure 2. Activation and inactivation of thiopurines TPMT.

TPMT polymorphisms are associated with the therapeutic efficacy and toxicity of mercaptopurines. Approximately 90% of the human population have high TPMT activity, about 10% have intermediate activity and 0.3% have low or no detectable activity. Eight TPMT alleles have been identified, and three of them account for about 95% of intermediate or low enzyme activity due to enhanced rate of proteolysis of the mutant proteins. The presence of these alleles (TPMT*2, TPMT*3A or TPMT*3C) is predictive of TPMT activity. Patients who are heterozygous for these alleles have intermediate activity, and homozygous are TPMT-deficient. The TPMT gene has been cloned and the most common variant allele responsible for low levels of activity among Caucasians encodes a protein with two alterations in the amino acid sequence as a result of SNP. These sequence changes result in a striking reduction in the quantity of TPMT at least because the variant protein is degraded rapidly. There are large differences in the types and frequencies of TPMT alleles among ethnic groups. 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 [3,10]. Also, patients who are heterozygous at the TPMT locus are at intermediate risk of dose-limiting toxicity [11,12]. By using PCR assays to detect the three signature mutations in these alleles it is possible to identify more than 90% of all mutant alleles [13,14]. In summary, these results can be used to determine safe starting doses for thiopurine therapy, which is an example of the individualization of therapy based on pharmacogenomic data.

5-Fluorouracil

5-FU and the oral prodrug capecitabine are uracil analogs that are widely used in the treatment of solid tumors, such as colorectal and breast cancer. 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

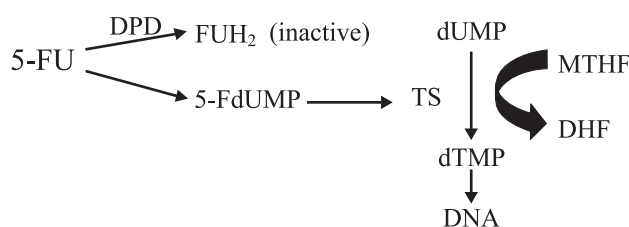


Figure 3. Activation and inactivation of 5-fluorouracil.

synthesis (Figure 3).

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) [15-17]. 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 treatment [10]. However, DPD is the initial and rate-limiting enzyme in the catabolism of the pyrimidine bases uracil and thymine, and it also catalyzes the reduction of 5-FU to the inactive metabolite dihydrofluorouracil (Figure 3). The molecular basis of DPD deficiency is complex. Thus far, more than 30 mutations in the DPD gene have been described, at least 20 of which have been reported to be functional [3,18]. In the general population, 3-5% of individuals are heterozygous carriers of mutations that inactivate DPD, and 0.1% are homozygous for mutations that inactivate DPD [19-21]. 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 nonfunctional protein, which is associated with severe toxicity and fatal outcomes of 5-FU treatment in some studies [15,16,22]. But, DPYD*2A is not the only mechanism for severe 5-FU toxicity. It is shown that many patients with severe 5-FU toxicity didn't have mutations in the coding region of the DPYD gene. So, the complexity of the molecular mechanisms controlling DPD activity *in vivo* complicate the application of DPD pharmacogenomics for prospective identification of patients likely to suffer severe 5-FU toxicity.

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 [23,24]. The expression of TS is partly controlled by a polymorphism within the 5'-promoter enhancer region, the so-called TSER of the TS gene, consisting of tandem repeats of 28-bp. Alleles containing two, three, four, five and nine copies of the repeated sequence have been described (TSER*2, TSER*3, TSER*4, TSER*5, TSER*9) with TSER*2 and TSER*3 being predominant alleles in studied populations [25,26]. Multiple *in vivo* studies have shown that increasing the number of repeats leads to an increase in TS mRNA levels and protein expression. For example, patients homozygous for the TSER*3 genotype are found to have a 3.6-fold elevated intratumoral TS mRNA levels [27], elevated TS protein levels [28], and poorer response rates [27,29,30] after 5-FU chemotherapy, compared to TSER*2 homozygous; and 1.7-fold increase between patients homozygous for TSER*3 and patients heterozygous for TSER*2/TSER*3. Finally, studies suggest that combined genotyping of DPYD and TSER functional variants might be very useful in prospectively selecting patients likely to respond 5-FU therapy. This is particularly important because of the availability of other antitumor agents (irinotecan, oxaliplatin), which can be used in combination with or instead of 5-FU if high TS or deficient DPYD are found.

Irinotecan

Irinotecan is a topoisomerase I inhibitor approved for use as first-line therapy for metastatic colorectal cancer in combination with 5-FU [31]. Irinotecan 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 (Figure 4a).

In humans, 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. Fatal events during single-agent irinotecan treatment have been reported [32], but concerns have been expressed because of an excessive rate of early deaths in colorectal cancer patients receiving irinotecan + fluorouracil regimens [33]. The risk of severe toxicity might be predicted by investigating the genetic variation of irinotecan disposition. 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 [34-36]. This genetic variation has been investigated in

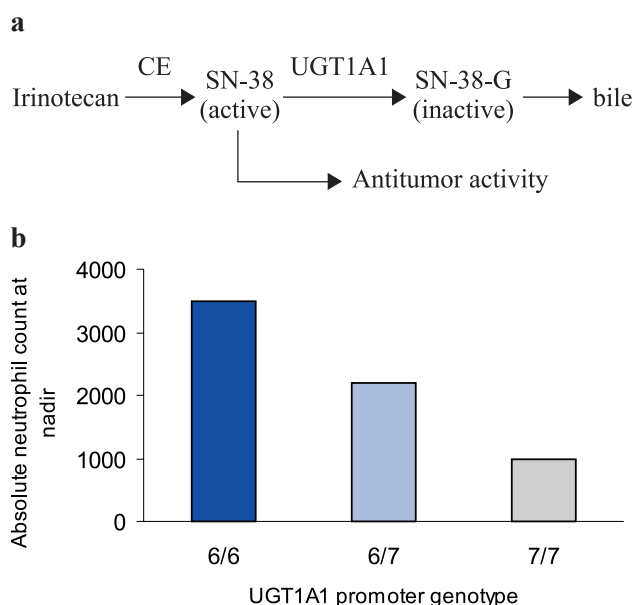


Figure 4. Activation and inactivation of irinotecan (a). Correlation of UGT1A1 genotype and neutrophil count (b).

relation to hyperbilirubinemic syndromes, because UGT1A1 enzyme catalyzes bilirubin glucuronidation [37]. Gene transcriptional efficiency has been inversely correlated to the number of TA repeats in the TATA box (5-8 repeats) [38]. A six-repeat allele is the most common. The presence of seven repeats results in the variant allele UGT1A1*28. This allele is associated with reduced UGT1A1 expression, which leads to reduced SN-38 glucuronidation [34-36]. For example, it was found a 3.85-fold increase in SN-38 glucuronidation in liver microsomes from individuals homozygous for six repeats compared to homozygous for seven repeats [39]. Also, it was recently shown that the UGT1A1*28 allele leads to significantly increased amounts of the active metabolite SN-38, and an increased chance of developing diarrhea and neutropenia during irinotecan therapy [39]. In a study of 20 patients with solid tumors treated with irinotecan, severe toxicity was only reported in UGT1A1*28 heterozygotes and homozygotes [39]. This study also showed that UGT1A1 promoter genotype is significantly correlated with absolute neutrophil count and nadir (Figure 4b). Finally, 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

Platinum analogs (cisplatin, carboplatin and oxaliplatin) are widely used in the treatment of solid tumors

such as testicular, lung, ovarian, breast and gastrointestinal cancers. These agents inhibit cellular replication by forming inter and intrastrand helix-deforming DNA adducts. The success of platinum complexes in killing tumor cells depends mainly on their ability to form various types of DNA adducts [40]. DNA adduct is a complex that forms when a chemical compound binds to DNA. As a result, accumulation of platinum agents leads to inhibition of DNA replication. 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 [41,42]. 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 [43]. A nonsynonymous SNP, altering a lysine to glutamine at codon 751 of the XPD protein was shown to be significantly associated with treatment outcome. 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 [44]. Twenty-four percent 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 [44].

- The XRCC1 gene has an important role in base excision repair. The enzyme encoded by this gene is 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. A higher percentage of patients with the arginine/arginine genotype responded to therapy, whereas the presence of either glutamine/glutamine or glutamine/arginine genotype was seen more in nonresponders [45]. The functional consequence of this arginine to glutamine alteration is decreased DNA repair capacity [46]. How-

ever, the sample sizes in these studies are small, so larger studies are needed to confirm these findings.

- 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 [47]. There are five subclasses of GST family (GSTA1, GSTP1, GSTM1, GSTT1 and GSTZ1) [48] that influence cytotoxicity to a variety of chemotherapeutic agents [49]. Stoehlmacher et al. [50] 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 [51]. 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 and sulfotransferase

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 [52]. 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 [53]. 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) [54]. In a recent retrospective study it was shown 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) [55]. Among women who didn't receive tamoxifen, association between survival and SULT1A1 genotype wasn't found. However, the result of this study is counterintuitive. Since the SULT1A1 isoform is involved in the hepatic cytosolic trans-selective sulfation of 4-OH-tamoxifen isomers [53], there might be expected higher circulating levels of 4-OH-tamoxifen for women homozygous for the low-activity

allele, and greater antitumor effect. Because the study investigated SULT1A1*2 polymorphism on the adjuvant breast cancer treatment, it was not possible to evaluate tumor response rates. So, patients with metastatic disease and with the low-activity allele receiving tamoxifen might be expected to have higher response rates but greater risk for toxicity. Finally, further prospective studies evaluating the influence of the SULT1A1 pharmacogenetics on clinical outcomes are needed to clarify and confirm these findings.

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's intake and proper activities of the 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 a polymorphic enzyme, that irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which 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 [56,57].

Methotrexate (MTX) is an antifolate chemotherapeutic drug used in the treatment of lymphoma, certain forms of leukemia and in patients undergoing bone marrow transplantation to prevent graft-versus-host disease. It is also given to treat some forms of cancers of the uterus, breast, lung, head, neck, and ovary. MTX is also given to treat rheumatoid arthritis when other treatments have been ineffective, and is sometimes used to treat 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 [58]. Interestingly, in patients with MTHFR 677CT alleles, increased risk of MTX-induced toxicity was reported only after low-dose MTX [58], 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

These examples represent situations in which a small number of genes 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 research is needed. Recently, two experimental approaches have been applied to the field of cancer pharmacogenomics: microarray analysis and the study of animal model system. 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. By using microarrays, 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, and for discovering new classes of tumors.

The future of cancer pharmacogenomics

Pharmacogenomics has great potential to revolutionize cancer therapy. Although there was substantial

success in situations where single genes have a large role in overall drug response, the future of cancer pharmacogenomics lies in whole-genome approaches. Microarray has shown great chances for individualizing cancer therapy in two ways: either through better diagnosis of subgroups with risk, or by direct markers of chemosensitivity. Finally, further improvements in technology are needed and expected to increase the application of this method in the clinical practice and reduce the overall expenses.

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