The impact of genomics and proteomics in the clinic: functional genetic polymorphisms and their value in response and toxicity prediction in solid tumours

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Introduction

Currently, type and intensity of chemotherapy is based on results from phase II–III studies and on patient’s body surface. Great efforts have been dedicated to identifying patients with increased chances of responding to treatment, and many factors have been associated with efficacy such as interactions with other drugs, histological differentiation of the tumour, age, gender, severity of the disease and others. In general, chemotherapeutic regimens have a narrow therapeutic index and administration of maximum doses to achieve the best response may put some patients at increased risk for severe side effects.

The hereditary basis of inter-individual differences of drug effects is variation in DNA, the genetic polymorphisms. Several types of polymorphisms including insertions, deletions, tandem repeats and single nucleotide polymorphisms (SNP) have been identified. The rapidly growing field of pharmacogenetic and pharmacogenomic studies constantly reveals new insights into efficacy and toxicity management of certain drugs by the individual patient.

This review reports on pharmacogenetics in solid tumours, including different cytotoxic agents, biologicals and tyrosine kinase (TK) inhibitors.

Principles of pharmacogenetics

The field of pharmacogenetics focuses on functional variations of those enzymes involved in metabolism of administered drugs. It has been recently discovered that variations in DNA sequence, so called polymorphisms, cause differences in protein structure and function. Pharmacogenetic approaches evaluate whether genetically determined variations of enzyme function translate into clinically significant differences in drug metabolism. Polymorphisms are diallelic markers.

The analysis of polymorphisms can result in three possible events: homozygous for allele 1, homozygous for allele 2, or heterozygous. The determined status of a polymorphism is known as the genotype. In case of a moderate or weak impact of one allele for the overall function, only the genotype homozygous for the polymorphism of interest may cause a significant alteration of function.

Pharmacogenetic investigations aim to explore associations between known functional polymorphisms and tumour response and toxicity as clinical endpoints. Due to the fact that alterations of the genetic information are a common phenomenon in solid tumours, analyses of the tumour genome is essential if tumour response is evaluated. DNA from normal tissue should be used when exploring a polymorphism’s impact on toxicity.

Current pharmacogenetics

Standard cytotoxic drugs in solid tumours include fluoropyrimidines, irinotecan, cisplatin and oxaliplatin, taxanes, gemcitabine and others. The majority of pharmacogenetic information available focuses on these drugs, because the biologicals bevacizumab and cetuximab have been introduced into clinical practice only recently.

Fluoropyrimidines

The fluoropyrimidines still remain as standard cytotoxic agent in the treatment of gastrointestinal (GI) tumours. Thymidylate synthase (TS), the target enzyme of 5-fluorouracil (5-FU), dihydropyrimidine dehydrogenase (DPD), the main player of 5-FU catabolism in the liver and methylentetrahydrofolate reductase (MTHFR), directly linked to the TS reaction have been investigated in more detail.

Three polymorphisms within the TS gene have been identified to alter TS expression. A 28-bp repeat polymorphism and a G/C SNP, both located within the 5’-region of the gene [1–4], significantly impact TS mRNA levels, which have been shown to correlate with response to 5-FU chemotherapy [5]. Horie et al. [1] first demonstrated an increased TS mRNA level in association with a 3-fold repeat of the 28-bp sequence (3R) compared to only a 2-fold repeat (2R) in vitro. These data were confirmed by Pullarkat et al. [2] and others human tissues [6]. Pullarkart et al. also introduced the idea of linking TS polymorphism and clinical outcome directly. Among 50 colorectal cancer patients the authors showed a significant higher response rate for TS 2R/2R carriers [2]. In conjunction with these data Iacopetta et al. [7] revealed that Dukes C colorectal cancer (CRC) patients possessing a TS 2R/2R or 2R/3R genotype benefited from an adjuvant therapy with
5-FU, but patients with TS 3R/3R genotype did not increase survival time despite chemotherapy. In contrast, Hitre et al. [8] described the exact opposite association between TS 28-bp repeat polymorphism and survival following adjuvant 5-FU chemotherapy in a recent paper analysing 166 CRC patients. Reviewing these data it has to be realised that Hitre et al. [8] used DNA from peripheral blood mononuclear cells as compared to Iacopetta et al. [7], who used tumour DNA. Considering the high rate of loss of heterozygosity (LOH), >50%, in CRC tumours regarding the TS gene locus [9], these different sources might significantly impact the analysis.

Almost simultaneously Kawakami et al. [3] and Mandola et al. [4] discovered an additional G/C SNP exclusively located within the middle repeat of a 3R TS variant. This SNP disrupts a binding domain for the transcription factor USF-1, thereby transforming a TS 3R into a TS 2R variant with regard to its function. For further pharmacogenetic analyses involving the TS gene this newly identified SNP should be considered. Recently Morganti et al. [10] confirmed a significant association between both TS gene polymorphisms and TS expression in colonic mucosa. Marcuello et al. [11] were the first to demonstrate a significant impact of this additional G/C SNP in the TS gene. Among 89 patients with CRC, who received a fluoropyrimidine-based chemotherapy the polymorphic sequence within the 5'-region of the TS gene predicted response only if both polymorphisms were considered [11]. The group comprising TS genotypes associated with low TS expression (2R/2R, 2R/3RC, 3RC/3RC) demonstrated a significantly improved response rate. Considering both TS polymorphisms, Gockkurt et al. [12] observed a trend for improved survival among gastric cancer patients treated with 5-FU combination chemotherapy when they possessed one of the low TS expression genotypes.

Pullarkat et al. [2] and recently also Lemote et al. [13] further describe significant correlations between TS polymorphism of the 5'-region of TS and fluoropyrimidine toxicity. Patients with TS 3R/3R, a 3R/2R or a 2R/2R genotype had a grade 3 or 4 toxicity rate of 3%, 18% and 43%, respectively (P < 0.01) among 90 CRC patients analysed.

In addition to the 5'-region polymorphisms a 6 bp deletion within the 3' UTR of the TS gene is linked to TS mRNA expression [14], although this association is contrasted by the findings of Calascibetta et al. [15], who did not find an link between the polymorphic region of the TS 3'-UTR and TS expression.

In conclusion, there is evidence that polymorphisms of the TS gene may predict response, survival and toxicity in GI cancer treated with fluoropyrimidines. However, some of the existing data are conflicting. Further investigations in larger groups of patients are needed to judge on the predictive value of TS polymorphisms for the clinic. Therefore, the polymorphism data should be compared with results from immunohistochemistry and gene expression analyses.

MTHFR controls intracellular 5,10-methylenetetrahydrofolate (CH\textsubscript{2}FH\textsubscript{4}) levels by irreversibly converting CH\textsubscript{2}FH\textsubscript{4} into 5-methyltetrahydrofolate. Depending on MTHFR activity, more or less substrate for the TS reaction will be provided. Two polymorphisms of MTHFR have been reported to determine enzyme activity: MTHFR-C677T and MTHFR-A1298C. In theory, a mutated MTHFR-677T variant or a MTHFR-1298C variant decreases MTHFR activity and presumably increases CH\textsubscript{2}FH\textsubscript{4} which is expected to correlate with improved response to 5-FU. In 2003, Cohen et al. [16] described in support of this theory a significantly increased response rate in CRC patients possessing at least one MTHFR-677T allele. This translated into an odds ratio for response to 5-FU of 2.86 (95% CI 1.06; 7.73) [16]. The association was confirmed by an analysis among 98 CRC patients by Etienne et al. [17] showing an increased response rate in MTHFR-677TT homozygotes compared to MTHFR-677CC carriers and by Jakobsen et al. [18] in a small prospective trial. A study in patients with locally advanced oesophageal cancer failed to demonstrate an association between MTHFR-C677T and clinical outcome [19]. One possible explanation may be the limited number of 68 patients and multimodal treatment consisting of radiochemotherapy +/- surgical resection. Less information is available for the MTHFR-A1298C polymorphism. A recent report by Etienne et al. revealed that, in contrast to the hypothesis, CRC patients homozygous for MTHFR-1298C had the worst prognosis [17].

Orotate phosphoribosyltransferase (OPRT) and DPD are involved in crucial steps of 5-FU activation and catabolism. OPRT catalyses the reduction of FUDP to FdUMP, an active TS-inhibiting metabolite. Recent data suggest a direct association between OPRT level and sensitivity to 5-FU [20]. A functional A213→G substitution of the OPRT gene increases activity of the enzyme. The A allele of this SNP in exon 3 was associated with toxicity augmentation in a small cohort (n = 52) of 5-FU treated CRC patients [21]. DPD catabolizes >80% of 5-FU in the liver [22]. More than 15 different mutations within the DPD gene are known to correlate with diminished activity of the enzyme [23]. The highest frequency of a known DPD variant with about 1% is reported for the G→A substitution in the invariant GT splice donor site flanking exon 14 (IVS14+1G→A) in Caucasians [24]. Although frequencies are low, consequences of impaired 5-FU catabolism have been shown to have fatal potential, caused by substantially increased hematopoietic and GI toxicity [25]. This should be considered, especially if other drugs with similar toxicity profiles such as irinotecan are added to the regimen.

Irinotecan

The use of the topo-isomerase I poison irinotecan (CPT-11) has become standard in the palliative setting of GI malignancies. The metabolic pathways of irinotecan are highly complex involving numerous enzymes for activation, transport and deactivation.

Irinotecan is transformed into its active player, SN-38, by tissue carboxylesterase (CE) mediated hydrolysis. Intestinal CE was demonstrated to be required for irinotecan activation in the GI tract [26]. Since GI toxicity is one of the use limiting toxicities of irinotecan, functional allelic variations of human CE-2 may become important modifying factors of irinotecan toxicity. However, the polymorphisms identified so far do not significantly alter protein activity [27].

Most pharmacogenetic analyses have been performed in the gene of hepatic uridine diphosphate glucosyl transferase (UGT) 1A1. UGT1A1 inactivates SN-38 by glucuronidation and its
activity is significantly altered by genetic polymorphisms. Diminished enzyme activity results from a 2 bp insertion (TA)₁-TAA (UGT1A1*28) in the promoter region [28]. Possession of at least one UGT1A1*28 allele was associated with a more than seven-fold increased chance of experiencing a grade 3 or 4 toxicity after irinotecan administration if compared to individuals homozygous for the UGT1A1 wild-type (odds ratio (OR) 7.2, 95% confidence interval (CI) 2.5, 22.3, \( P < 0.001 \)) [29]. Several smaller studies confirmed the link between UGT1A1 and irinotecan toxicity [11, 30].

A newly identified genetic variant (3263T→G) in the phenobarbital-responsive enhancer module of the UGT1A1 promoter lowers transcriptional activity of UGT1A1. However, an analysis among 119 irinotecan-treated patients could identify this polymorphism as an independent marker for irinotecan toxicity [31].

The additional hepatic enzyme UGT1A9 and the extra-hepatic form UGT1A7 significantly impact SN-38 glucuronidation [32]. A G→A substitution at position 766 (exon 1) of the UGT1A9 gene causes the amino acid substitution D256N, thereby reducing SN-38 glucuronidation efficacy to <5% if compared to the wild-type UGT1A9 [33]. This highly functional polymorphism should be incorporated into pharmacogenomic analyses of irinotecan toxicity and/or efficacy.

**platinum (cisplatin, oxaliplatin)**

The platinum drugs cisplatin and oxaliplatin are frequently administered in patients with GI malignancies. Platinum blocks DNA replication by formation of different types of adducts depending on the compound used. Members of the DNA repair family (e.g. ERCC1, ERCC2) and glutathione S-transferases (e.g. GSTP1) determine efficacy of platinum drugs.

The excision repair cross-complementing (ERCC) gene family is essential for the removal of platinum-mediated DNA adducts and gene expression data in CRC and gastric cancer confirm the inverse relationship between impaired DNA-repair capacity (e.g. low ERCC activity) and superior responses to platinum compounds [34, 35]. Consistently, polymorphisms that alter nucleotide excision repair (NER) function could be linked to clinical outcome in patients receiving platinum-based chemotherapy. A SNP within the ERCC2 (XPD) gene (codon 751) causes the amino acid exchange from lysine (Lys) to glutamine (Gln). An improved response rate of 24% was observed for patients possessing two 751Lys alleles compared to 10% in Lys/Gln or Gln/Gln carriers (\( P = 0.015 \)) [36]. The survival benefit for 751Lys homozygotes was reconfirmed in a study of 106 patients. All patients received a 5-FU/oxaliplatin combination chemotherapy [37]. A recent report by Vignier et al. [38] demonstrated a direct correlation between a functional polymorphism within the ERCC1 gene (codon 118) and response to 5-FU/oxaliplatin chemotherapy in CRC patients. Patients possessing a ERCC1 genotype containing a less-used coded 118 (AAT versus AAC) showed significantly increased response rates of 62% (AAT/AAT) and 42% (AAC/AAT) compared to only 21% in patients homozygous for the wild-type ERCC1 (\( P = 0.018 \)). Conversely, McLeod and colleagues could not verify the association for the ERCC2 polymorphism in a large cohort of patients that received oxaliplatin [39]. One reason for these differences may be that the role of the ERCC2 K751Q polymorphism in oxaliplatin metabolism is not completely understood since most exploring experiments involved cisplatin.

An additional factor for genetically determined inter-individual platinum efficacy and toxicity may arise from the action of glutathione S-transferases (GSTs). The isoenzyme GSTP1 appears to be predominantly expressed in GI tissues and especially in GI tumours at very high levels [40]. Biochemical experiments delivered information for direct involvement of GSTP1 in sufficient detoxification of cisplatin, and GSTP1 being responsible for both intrinsic and acquired resistance to platinum [41, 42]. The 313A→G SNP causing replacement of isoleucine (Ile) by valine (Val) in the GSTP1 protein diminishes substrate binding thereby leading to impaired GSTP1 activity [43]. In a retrospective analysis among patients that received 5-FU/oxaliplatin chemotherapy, those who harboured two Val alleles experienced the most favourable survival (median, 25 months, 95% CI 9.4, 25+ months) while the Ile/Ile genotype was associated with the least favourable survival (median, 7.9 months, 95% CI 5.4, 9.6 months). Intermediate survival outcome was noted for the Ile/Val carriers (median, 13 months, 95% CI 8.4, 24 months; \( P < 0.001 \)) [44]. Current data by Goekkurt et al. [12] in gastric cancer patients also demonstrated significantly different clinical outcome according to GSTP1 genotype. Among 5-FU/cisplatin-treated patients, those with a GSTP1 105Val genotype derived the highest benefit from the chemotherapy. The results by McLeod et al. [39] again contrasted the finding of a significant association between GSTP1 genotype and clinical outcome to oxaliplatin chemotherapy. A possible explanation may be the difference of chemotherapy regimen. The study by McLeod et al. [39] included only patients receiving first-line chemotherapy, while the retrospective analysis of Stoehlmacher et al. [44] was performed among patients that had failed at least one chemotherapy. The data were confirmed indirectly by Grothey et al. [45] who showed that patients possessing a Val-containing GSTP1 genotype experienced more and an earlier onset of oxaliplatin-induced neurotoxicity.

**other cytotoxic agents**

Gemcitabine and taxanes are also frequently administered in solid tumours. To date 14 different genetic variants have been identified in eight genes involved in the metabolic pathway of gemcitabine [46]. However, their impact on gene or protein function is currently not known. The cytochrome P450 system mediates the taxane metabolism. Kumar et al. [47] described hydroxylisation of taxanes by the CYP450 isoform CYP2C8 as major mechanism for pharmacological deactivation of the taxane. Based on these findings Dai et al. [48] recently reported that the polymorphic allele CYP2C8*3 including both Lys139Arg and Arg399Lys significantly impairs paclitaxel metabolism. Clearance of paclitaxel was two times lower in patients possessing a CYP2C8*7 Phe269Ile genotype as compared to those carrying a wild-type allele. Association studies between these polymorphisms and clinical parameters are not yet available.
inhibitors of epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF) and tyrosine kinases

Targeted therapies have been successfully introduced into the treatment schedules of solid cancers. Improved overall survival was demonstrated in CRC patients receiving drugs targeted at epidermal growth factor receptor (EGFR) [49] or vascular endothelial growth factor (VEGF) [50].

Recently, two different groups independently described an association between specific mutations within the EGFR gene and responsiveness to gefitinib in non-small cell lung cancer (NSCLC), a tyrosine kinase inhibitor targeting the EGFR [51, 52]. Mutations within the tyrosine kinase domain of EGFR were found in 89% (8/9) of patients responsive to gefitinib as compared to none among patients without response (0/7) (P < 0.001). These findings need confirmation in a larger cohort of patients. Interestingly, a lack of these EGFR mutations was reported in CRCs and neuroendocrine tumours, indicating less activity for TK inhibitors like gefitinib in these tumour entities.

A fascinating current report by Kobayashi et al. [53] illustrates both the potential of pharmacogenetic analyses for the development of targeted therapy and the power of available techniques to understand the genetics of cancer. A NSCLC patient with EGFR mutation initially showing complete remission to gefitinib for 2 years relapsed. Structural and biochemical studies discovered a second point mutation in the tumour tissue that led to gefitinib resistance.

No data are currently available linking DNA variations to GI cancer patients receiving EGFR targeted therapy. However, a pilot study among 39 patients with metastatic CRC who received cetuximab indicated a possible modulatory effect on survival by a polymorphism in the cyclin D1 (CCND1) gene [54]. Cyclins are involved in cell cycle arrest (G1 phase) after blockade of EGFR by cetuximab. A frequent polymorphism in exon 4 of the cyclin D1 gene results in alternative splicing with two different mRNAs. Significant differences according to survival were observed between genotype groups with heterozygotes showing the longest median survival (P < 0.05). Due to this result and the preliminary character of this pilot study the finding needs confirmation.

There are no reports of known functional polymorphic variants of EGFR in patients who received EGFR-inhibiting chemotherapy at this time, although associations between EGFR polymorphisms and response to 5-FU/oxaliplatin chemotherapy and with tumour recurrence after chemoradiation are reported [55, 56]. There are currently no pharmacogenetic data on bevacizumab.

**Conclusion**

Impairment of a single gene affecting drug metabolism, interactions with cellular targets or transport can cause a dramatic shift in the toxicity profile. Thus, pharmacogenetics (focusing on a single gene) may provide sufficient information to predict toxicity. In contrast, prediction of drug efficacy requires concurrent analysis of multiple variants (pharmacogenomics) due to its multifactorial nature. Analyses of high predictive value for drug response should include not only the individual’s genetic background, but also gene–gene interactions, somatic mutations of the tumour cell and dynamic characteristics such as variations in RNA expression. The individualisation of chemotherapy by a pharmacogenetic approach appears attractive. Pharmacogenetic association studies analysing toxicity and efficacy of chemotherapy in solid tumours are performed at a rapid pace. More importantly, incorporation of predictive markers in design of prospective clinical studies has started. We should increase our efforts in this field of translational medicine to tailor patients’ treatment in the near future.

Table 1 shows relevant genetic polymorphism to current treatments in solid tumours.

**Table 1. Genetic polymorphisms and mutations relevant to therapy in solid tumours**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Drug</th>
<th>Clinical implication</th>
</tr>
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<tbody>
<tr>
<td>TS</td>
<td>5′-region (28bp repeat, G/C SNP)</td>
<td>5-FU</td>
<td>Response, survival, toxicity</td>
</tr>
<tr>
<td>DPD</td>
<td>IVS14 + 1G→A</td>
<td>5-FU</td>
<td>Toxicity</td>
</tr>
<tr>
<td>OPRT</td>
<td>213G→A</td>
<td>5-FU</td>
<td>Toxicity</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>(TA)6/7TAA</td>
<td>Irinotecan</td>
<td>Toxicity</td>
</tr>
<tr>
<td>XPD</td>
<td>Lys751Gln</td>
<td>Oxaliplatin</td>
<td>Response, survival</td>
</tr>
<tr>
<td>GSTP1</td>
<td>ile105Val</td>
<td>Oxaliplatin &amp; cisplatin</td>
<td>Disease progression, survival</td>
</tr>
<tr>
<td>EGFR</td>
<td>Mutations in exon 18, 19, 21</td>
<td>TK inhibitors</td>
<td>Response</td>
</tr>
<tr>
<td>CCND1</td>
<td>A870G</td>
<td>Cetuximab</td>
<td>Survival</td>
</tr>
</tbody>
</table>

SNP, single nucleotide polymorphism; 5-FU, 5-fluorouracil; TK, tyrosine kinase.

**References**

5. Leichman L, Lenz HJ, Leichman CG et al. Quantitation of intratumoral thymidylate synthase expression predicts for disseminated colorectal cancer response and...


