REVIEW ARTICLE

Predictive diagnostics in colorectal cancer: impact of genetic polymorphisms on individual outcomes and treatment with fluoropyrimidine-based chemotherapy

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Abstract The 5-fluorouracil (5-FU)-based chemotherapy is a standard treatment for patients with colorectal cancer. However, a relevant number of patients suffer from severe toxic side effects, such as haemotoxicity, while lacking clinical response to adjuvant therapy. The inter-individual variations of drug toxicity and efficacy of the pyrimidine antagonist observed in clinical practice are mainly determined by genetic polymorphisms. The screening of genotypes, such as thymidylate synthase, dihydropyrimidine dehydrogenase, methylene tetrahydrofolate reductase, orotate phosphoribosyltransferase or glutathione S-transferase, could help identifying those patients with colorectal carcinoma who can actually benefit from a 5-FU-based therapy. The current chapter elucidates the roles of the polymorphisms in the enzymes involved in the 5-FU metabolic pathway as prognostic and predictive markers. It reports on the relationship between various genotypes in patients with colorectal carcinoma and their responsiveness to a 5-FU-based chemotherapy, and concludes with an outlook on possible future directions in treatment of colorectal cancer.

Keywords Thymidylate synthase · Dihydropyrimidine dehydrogenase · Methylene tetrahydrofolate reductase · Orotate phosphoribosyltransferase · Glutathione S-transferase · Clinical outcome · Individualised treatment · Predictive markers

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Introduction

Colorectal tumours are a leading cause of cancer death worldwide. Surgical resection of primary and regional lymph nodes is among the standard therapies for treatment of colorectal tumours. Although surgical resection alone is potentially curative in colorectal cancer patients, many patients develop local or distant recurrences [1].

In the 1950s, fluoropyrimidines including 5-FU were introduced into clinical trials. Twenty years ago, adjuvant treatment of colon cancer patients was validated and improved the curative rates. Numerous analyses have shown that 5-FU-based systemic adjuvant chemotherapies have improved survival in high risk patients. In adjuvant settings, 5-FU-based chemotherapies are currently an integral component of the management of colon cancer. However, its clinical application is limited, because 5-FU has gastrointestinal and hematological toxicity. The additional introduction of active drugs, such as oxaliplatin, bevacicumab or cetuximab, has provided clinical benefit for the patient in both early and late stages of the disease. Adjuvant therapy for colorectal cancer patients consists primarily of combinations of 5-FU/leucovorin with oxaliplatin or oral capecitabine. The combined regimens of 5-FU, leucovorin and oxaliplatin have been shown to prolong significantly disease-free survival. In the treatment of colorectal cancer patients the introduction of the platinum compound oxaliplatin has increased response rates up to 25%, even in heavily pretreated relapsing patients when administered in combination with 5-FU [2]. Also, the angiogenesis inhibitor bevacizumab and the epidermal growth factor receptor inhibitor cetuximab have shown activities when combined with 5-FU/leucovorin-based regimens as first-line treatment of advanced disease. For metastatic disease the 5-FU-based combination therapy with either irinotecan or oxaliplatin

plus bevacizumab has resulted in a significant improvement in response, and disease-free and overall survival. However, a relevant number of patients still get toxic side effects from the treatment while lacking a response by the tumour. In the worst cases, chemotherapy toxicity may accelerate death [3]. Moreover, the risk of recurrence is still high in many patients. Therefore, there is a need to tailor the therapy. Predicting an effective regime with minimal toxicity in the palliative situation might potentially improve the quality of care by allowing individualization of treatment. The dose of agent and the combination of 5-FU with other drugs should be adapted for patients at high risk.

Even with different doses and schedules, the response rate of metastatic colorectal cancer is only 21% when treated with 5-FU and leucovorin. To predict the clinical outcome of colorectal cancer patients treated with adjuvant 5-FU-based chemotherapy, endeavours have been made to investigate the efficacy, tolerability and toxicity of 5-FU, together with why some subgroups do and other groups do not benefit from a 5-FU-based therapy. Chemotherapy-based toxicity is caused by many factors such as organ dysfunction and genetic variability. In particular, polymorphic variations of the molecules involved in the metabolic pathway of 5-FU may

contribute to the differences in clinical outcome among patients. Therefore, it is of note to investigate the genotypes and to generate a pharmacogenetic profile that may help predicting the clinical outcome of a 5-FU-based chemotherapeutic regimen. Several types of polymorphisms affect the gene expression of enzymes in the metabolism of 5-FU and include insertions, deletions, tandem repeats and single nucleotide polymorphisms. To choose the effective regime with minimal toxicity and to limit treatment failure, DNA derived from different tissues, such as tumour tissues, leukocytes and blood may be used for the experimental approaches on the genotypes. Associations between the potential drug toxicity and the genetic patterns may be determined by using germ-line DNA. Since the primary tumour harbours tumour-specific aberrations, which would otherwise escape analyses, the drug efficacy may be examined by DNA derived from the primary tumour.

Polymorphisms of thymidylate synthase

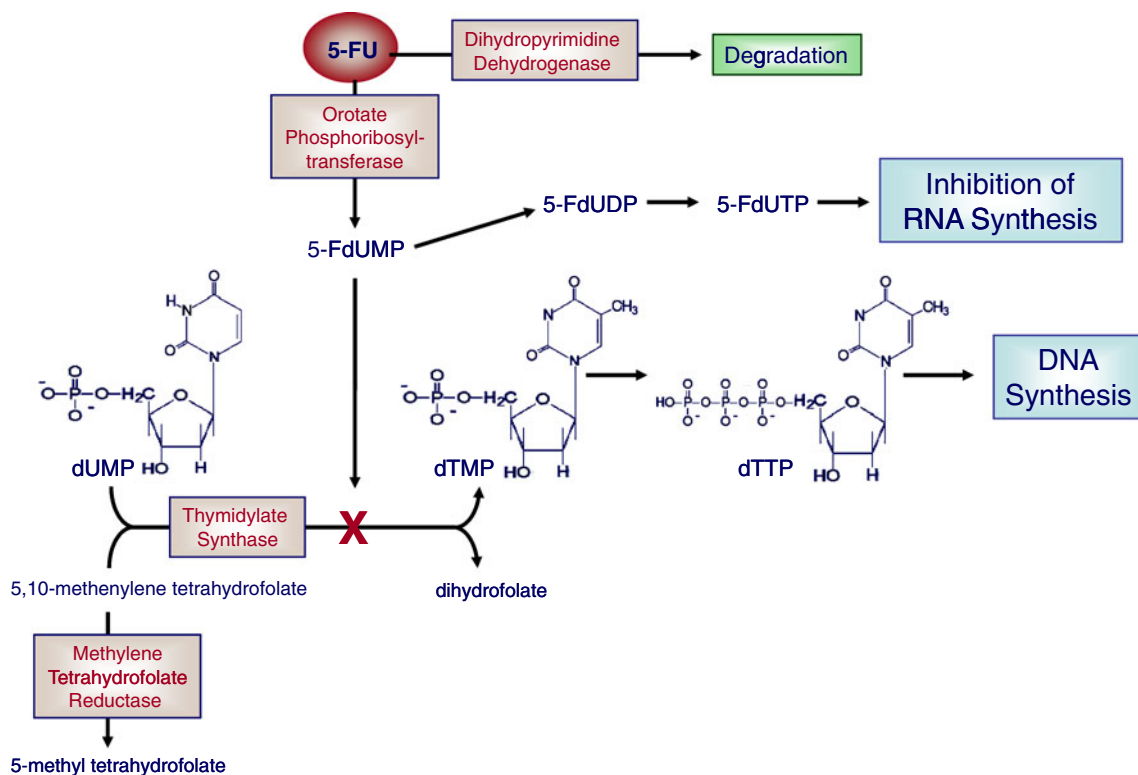As shown in Fig. 1, the action mechanisms of the fluoropyrimidine 5-FU are the inhibition of thymidylate

Fig. 1 The 5-fluorouracil (5-FU) metabolism pathway. In tumour cells 5-FU is converted to 5-fluorodeoxyuridine monophosphate (5-FdUMP) by the action of orotate phosphoribosyltransferase. 5-FdUMP inhibits the DNA synthesis by competing with deoxyuridine monophosphate (dUMP) for binding to thymidylate synthase in a complex that is stabilized by the reduced folate 5,10-methylene tetrahydrofolate. 5-FU

can also inhibit RNA synthesis in a pathway that involves its metabolism by orotate phosphoribosyltransferase to 5-fluorouridine monophosphate (5-FUMP) and subsequent conversion to 5-fluorouridine triphosphate (5-FUTP) via 5-fluorouridine diphosphate (5-FUDP). Extracellular catabolism of 5-FU by dihydropyrimidine dehydrogenase reduces the level of 5-FU for the uptake into the tumour cells

synthase (TS) by 5-FdUMP and the metabolic impairment of RNA by incorporation of 5-FdUTP into RNA [4]. Based on its polymorphisms and expression levels, TS has been described to be a predictive factor for the efficacy of the 5-FU treatment [5, 6]. As a key enzyme it regulates the dTMP levels by catalyzing the reductive methylation of dUMP by 5,10-methylene tetrahydrofolate. In this rate-limiting reaction, dTMP and dihydrofolate are formed. The thymidylate biosynthesis is the only *de novo* source for dTMP which is a precursor nucleotide for DNA replication. 5-FdUMP, an active metabolite of 5-FU binds to TS, and causes the formation of a stable ternary complex which suppresses the enzyme activity of TS [7]. As a result, dUMP molecules accumulate along with a decrease in dTMP levels. After conversion of dUMP into dUTP, dUTP replaces dTTP as a substrate for DNA polymerases and is incorporated into the newly synthesized DNA strand. Uracyl-N-glycosylase actually detects incorrectly incorporated uracil bases in the DNA strand, e.g. after the spontaneous deamination of deoxycytidine. Although it removes the incorporated uracil bases, the new DNA strand is incorrectly repaired by dUTP or 5-FdUTP, because dTTP is not available.

Since TS is required for the delivery of nucleotides used in DNA replication, and consequently, for tumour cell proliferation and tumour growth, numerous reports have engaged in studying the polymorphisms of TS gene, and demonstrated that the intratumoural yields of TS mRNA transcripts and protein molecules may be a predictor of response and survival to 5-FU-based chemotherapy [8–10]. Whereas low TS mRNA levels may associate with a better response to 5-FU-based chemotherapy and improve 5-year survival, higher expression levels may fail to respond to this treatment [11, 12]. However, the minor TS levels may also enhance the cytotoxic side effects of 5-FU provoking the determination of patient therapy [9, 13]. These observations suggested that screening of TS genotype that is linked with TS expression might have the potential to identify those patients with colorectal carcinoma who benefit from a 5-FU-based adjuvant therapy. So far, three functional polymorphisms of the TS gene have been identified: variable number tandem repeat (VNTR), single nucleotide (SNP) and deletion (1494del6) polymorphisms, which significantly affect TS gene expression.

With respect to the VNTR polymorphism, the 5′ regulatory region of the TS promoter contains a variable number of a 28-bp repeat (R), mainly, double (2R) or triple (3R) repeats. These tandem repeats contain E-boxes for the transcription factor USF-1 (upstream stimulatory factor-1) that activate transcription of the TS gene by binding to these motifs [14]. Accordingly, the higher number of repeats is associated with a higher promoter activation and transcription. It was reported that of the three VNTR groups, including 2R/loss, 2R/3R and loss/3R, the poly-

morphism 2R/loss exhibited the lowest mRNA expression level, the best response to 5-FU and the longest medium survival time [15].

A further polymorphism is a G/C (guanine/cytosine) substitution of the 12th nucleotide in the second repeat of the triple repeat variant (3RC/3RG). The presence of the SNP in the USF-1 binding site causes a decrease in transcriptional activity of the *wild type* triple repeat (3RG/3RG) variant which is comparable with the 2R/3RG variant [16]. Functional analysis showed that a plasmid construct encoding for the 3RG/3RG variant had 3–4 times higher translation efficiency than other polymorphic sequences [17].

In addition, a 6-bp deletion (1494del6) polymorphism was identified in the 3′ untranslated transcriptional region (3′UTR) of the TS gene. An association between this deletion (del) and the mRNA instability has been described [18]. To investigate the function of the 1494del6 polymorphism *in vitro*, a luciferase system was used, and showed a ~70% decrease in luciferase activity and mRNA levels. The TS 3′UTR construct containing the heterozygous del/6 bp deletion also had a higher rate of mRNA degradation compared to the *wild type* (6 bp/6 bp) construct [18]. As examined in studies by Kawakami et al. [19] and Uchida et al. [20], the deletion at the TS locus in tumour cells may affect the actual expression level. It seems that TS promoter and 3′UTR polymorphisms are dependent on one another, because disequilibrium between both polymorphisms may be a general feature of the TS gene [21, 22]. Conversely, Merkelbach-Bruse et al. disproved an association between microsatellite instability (MSI) status and TS expression. Based on a higher mRNA stability, they observed that the 3R/3R or 2R/3R genotypes of TS had higher mRNA levels whereas polymorphic variants of the 3′UTR did not influence the TS mRNA level [23].

The TS gene is localized on the short chromosomal arm 18. Vogelstein et al. reported that ~40% of patients with colorectal tumours had deletions in this region [24]. Other laboratories even found losses of heterozygosity (LOH) in more than half of the examined carcinomas [25]. These patients possess an imbalance of the TS allele that remains occult on examining exclusively the genotype of the leukocyte (*wild type*) DNA. Frequent LOH at the TS locus was detected in 62% of the informative cases by RT-PCR using TS mRNA and microsatellite analyses of the marker D18S59 located downstream of the TS gene sequence [19]. These high frequencies of DNA losses support an unstable TS genotype during carcinogenesis. It is of note that in respect of the repeat number of the heterozygous genotype (3R/2R) bearing LOH, the cancer tissue with the genotype 2R/loss expressed a significantly lower level of TS protein than did the tumour with the genotype 3R/loss [19]. These data indicate a significant difference between the TS genotypes of tumour and normal tissues.

In respect to the different TS variants, discrepancies in risk of toxicity, clinical outcome and gene expression have been reported, because most studies have considered only a single TS polymorphism, [16]. Moreover, it should be critically noted that genotyping of the TS variants in numerous analyses was performed by using normal leukocyte DNA instead of tumour DNA indicating that the occurrence of LOH was not considered [26]. A recent study explored the effect of the three polymorphisms of the TS gene on overall and progression-free survival of colorectal cancer patients subjected to 5-FU-based chemotherapy and showed that the 3RG/3RG 6 bp/6 bp combined genotype (*wild type*, high expression) identified a subgroup of advanced patients with poor prognosis. However, none of the studied polymorphisms alone affected overall or progression-free survival [27]. A further study investigated the three polymorphisms of the TS gene and the response to a 5-FU-based chemotherapy in patients with colorectal cancer and liver metastases. The distinction between high (2R/3G, 3RC/3RG and 3RG/3RG) and low (2R/2R, 2R/3RC and 3RC/3RC) TS expression genotypes referred to a significant association with tumour response [28]. Tumour recurrence is a major problem after curative resection in the management of colon cancer therapy. Lurje et al. showed that high-expression variants of TS may help to identify stage II and stage III colon cancer patients who are at high risk of developing tumour recurrence, and also those who are more likely to benefit from a 5-FU-based adjuvant chemotherapy. Patients harbouring the TS 3RG 6 bp haplotype were at highest risk in developing tumour recurrence. In both univariate and multivariable analyses, TS 3RG alone or in combination with TS 1494del6 proved to be adverse prognostic markers [29].

Numerous reports have not only argued for, but also against, the incidence of diverse polymorphisms occurring with a better clinical response and overall survival of the patients [9, 30–32]. A recently published research showed that the different polymorphisms of the TS gene (SNP and VNTR) may not be related with disease-free survival in patients with stage III colorectal cancer receiving adjuvant chemotherapy [33]. In our study, we investigated whether the presence of single polymorphisms (VNTR, 1494del6 and SNP), LOH at the microsatellite markers D18S59, D18S1140 and D18S976 mapped up- and downstream to the TS locus, and combinations of them at the TS locus in leukocytes, primary tumour and blood of patients with advanced colorectal carcinoma who received 5-FU-based chemotherapy, as well as their increased DNA concentrations in blood, were associated with the clinical and histopathological risk parameters of these patients. Apart from the significant correlations of the cell-free serum DNA concentrations with the 1494del6 and combined polymorphisms, and of the age with VNTR and VNTR plus

1494del6, the combined polymorphisms detected in blood serum only tended to associate with the response to therapy by the patients. Moreover, the borderline significance detected in serum showed rather an inverse correlation to the number of polymorphisms and a low impact on response to 5-FU. Our findings provided an insight into the molecular diversity of the regulation of TS gene expression, and suggested that the variance in responses to 5-FU-based chemotherapy cannot consistently be explained by the genetic alterations detected in the TS gene [34]. Although numerous *in vivo* and *in vitro* studies have described the association between TS genotype with TS protein level [9, 14, 17], a recent report could not find such correlations by immunohistochemistry. This laboratory also showed that the immunohistochemistry data did not significantly associate with the clinical outcome of patients, and that VNTR was the only polymorphism of the TS gene demonstrating a significant association with later recurrence and cancer-specific survival. Assessment of the two other polymorphisms (SNP and 1494del6) did not deliver any additionally prognostic information [35].

Summing up, these studies show that the prognostic value of TS genotypes in patients receiving adjuvant 5-FU-based chemotherapy is still a matter of controversy. They also reflect the complexity of TS gene regulation and that other enzymes involved in thymidylate biosynthesis should also be considered.

Polymorphisms of orotate phosphoribosyltransferase

As shown in Fig. 1, 5-FU is preferentially phosphorylated by the orotate phosphoribosyltransferase (OPRT) to produce the nucleotide 5-FdUMP. 5-FdUMP is then phosphorylated to 5-FdUDP, which can be either reconverted to 5-FdUMP or phosphorylated to the active nucleotide 5-FdUTP. Now, 5-FdUTP can be extensively incorporated into RNA (F-RNA), disrupting normal RNA process and function [36]. An inhibitor of OPRT is potassium oxonate which could lower the levels of 5-FdUMP leading to a decrease of ~70% in F-RNA synthesis in the small intestine and a reduction in gastrointestinal toxicity [37].

In mucosal carcinoma and infiltrative lesions the expression of OPRT correlated significantly with the presence of lymphovascular invasion suggesting that OPRT is involved in the invasion and metastasis of colorectal tumours [38]. On the other hand, using immunohistochemistry, Tokunaga et al. found that a high OPRT expression in stages II–IV disease was correlated with a better overall survival [39]. Common polymorphisms, including SNPs 213G/C (guanine/cytosine substitution at position 213), 638G/C, 1050T/A (thymine/adenine) and 1336A/G (adenine/guanine), have been identified in the coding region of OPRT [40]. The SNP 213G/C of OPRT may be a significant predictor of 5-FU-induced

toxicity in patients treated with 5-FU adjuvant therapy, and have an independent value for prediction of severe grade 3 to 4 diarrhea [41]. In contrast, the SNPs 638G/C, 1050T/A, and 1336A/G of OPRT may have no major influence on 5-FU sensitivity [42].

Polymorphisms of dihydropyrimidine dehydrogenase

The systemic exposure to 5-FU is fundamentally determined by the genetically polymorphic enzyme dihydropyrimidine dehydrogenase (DPD). DPD is the initial and rate-limiting enzyme in the three-step catabolic pathway of 5-FU (Fig. 1). More than 80% of administered 5-FU is degraded by DPD in human liver. Deficiency in the DPD enzyme activity caused a delay in the clearance of 5-FU from blood and the accumulation of 5-FU [43]. As a result, chemotherapeutic toxicity of 5-FU has been associated to low levels of DPD enzyme activity. At least 50% of patients who had highly toxic side effects were genotypically heterozygous or homozygous for SNPs in the DPD gene [44, 45].

As determined by immunohistochemistry and/or RT-PCR, analyses on DPD expression in primary colorectal tumours of patients treated with 5-FU-based chemotherapy showed either significant associations of a low DPD expression with prolonged overall and disease-free survival [46, 47] or no associations [48]. Accordingly, immunohistochemistry of paraffin-embedded primary tumour tissues demonstrated that patients with high DPD expression levels had significantly shorter disease-free and overall survival than patients with low DPD levels. In combined analyses, including the determination of TS and DPD expression, patients with low TS and DPD had the best outcome in terms of disease-free and overall survival. This retrospective study suggested that the combined assessment of TS and DPD might be useful in evaluating the prognosis of patients with Dukes' B and C colon carcinoma receiving 5-FU adjuvant chemotherapy [46]. However, conflicting results have been reported in metastatic patients [39, 49, 50].

Although the precise role of DPD as a prognostic factor in the efficacy of 5-FU still has to be established, there is evidence that patients with a DPD deficiency are prone to the development of severe 5-FU-associated toxicity. The exon 14-skipping mutation (IVS14+1G/A mutation) provokes a changed splice site leading to an aberrant protein sequence and a loss of the catalytic activity of DPD. This gene variant accounted for 24–28% of all patients suffering from severe 5-FU toxicity [51]. In the cofactor binding domain and the electron transport domain of DPD the 2846A/T polymorphism leads to an amino acid substitution and a reduced enzyme activity. This gene variant has been detected in 2% of the population [52]. To tailor 5-FU administration and determine strategy in first-line chemo-

therapy of advanced colorectal cancer, Capitain et al. investigated the polymorphisms of DPD and their influence on tolerance and toxicity in patients initially treated with 5-FU. They detected that the toxicity was significantly linked to the SNP 2846A/T and the splicing mutation IVS14+1G/A of DPD [53]. In five out of eight cases with severe side effects, Amstutz et al. observed that a potential association existed between a haplotype containing three intronic polymorphisms (IVS5+18G/A, IVS6+139G/A and IVS9+51 T/G) and a SNP (1236G/A). Comparing their data with those from other studies, they suggested that the relative importance of particular DPD mutations (IVS14+1G/A and 2846A/T) for predicting severe 5-FU toxicity differs geographically across Europe [54]. Moreover, numerous further polymorphisms in the DPD gene, such as 85T/C, 496A/G, 1601G/A and 1627A/G, have been identified but their effect on transcription and their influence on clinical outcome of colorectal cancer patients treated with 5-FU are still unclear [55].

Polymorphisms of methylene tetrahydrofolate reductase

5,10-methylene tetrahydrofolate reductase (MTHFR) is a key enzyme in the regulation of intracellular folate levels. It affects both, DNA synthesis and methylation. In a one-carbon metabolism it catalyses the irreversible conversion of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate to generate the folate derivative for homocysteine remethylation to methionine (Fig. 1). In human cancer cells the inhibition by specific antisense RNA molecules against MTHFR affected tumour growth, and 5-FU could potentiate this significant inhibitory effect [56].

Two common SNPs (677C/T and 1298A/C) have been described to reduce MTHFR enzyme activity and contribute to an alteration of intracellular folate distribution. The 677C/T transition in exon 4 results in an alanine to valine substitution in the predicted catalytic domain of MTHFR. This substitution made the enzyme thermolabile, and reduced ~70% and ~35% of its enzyme activity in homozygotes and heterozygotes, respectively [57]. The 1298A/C transition in exon 7 results in a glutamate to alanine substitution within the regulatory domain of the protein and led to a decreased enzyme activity, albeit not to the same extent as that of the 677C/T allele [58]. Since a decrease in MTHFR enzymatic activity may lead to a decrease in intracellular 5-methyl tetrahydrofolate concentrations, it is presumed that tumours exhibiting the rare MTHFR variants may be more sensitive to 5-FU cytotoxicity than patients with a wild-type genotype. Moreover, the concomitantly higher concentrations of 5,10-methylene tetrahydrofolate may in turn favour the formation and stability of the inhibitory ternary complex consisting of TS, 5,10-methylene tetrahydrofolate and 5-FdUMP.

Several clinical studies have investigated the potential predictive role of these genetic variants in toxicity and efficacy of 5-FU, but contradictory data have been published [59]. Fernandez-Peralta et al. examined the relationship of 677C/T and 1298A/C polymorphisms with biological, clinicopathological, genetic and epigenetic features of the colorectal tumours and showed that both polymorphisms were relevant and independent factors of patient outcome after 5-FU-based treatment. The variant 677C/T had a protective effect on colorectal cancer development, whereas the variant 1298A/C did not produce any effect on disease risk [60]. Moreover, both polymorphisms were reported to be associated with a sex-specific clinical outcome in metastatic colon cancer patients treated with FU-based chemotherapy. The 1298A/C polymorphism in MTHFR correlated statistically significantly with the overall survival of female patients with metastatic colon cancer, but no such correlation was observed for male patients. Therefore, it was suggested that this variant might be a prognostic marker for female patients with metastatic colon cancer [61]. These findings have been supported by another study showing that the overall survival was higher for C/C female patients than for A/C female patients [62]. Etienne et al. evaluated the MTHFR polymorphisms in patients with metastatic colorectal cancer who were treated with 5-FU and leucovorin. Their response rate was significantly associated with the 677C/T genotype, but not connected with the 1298A/C genotype [63]. In the study by Jakobsen et al. the response rate was 66% for 677T/T compared with 33% and 21% for 677C/C and 677C/T, respectively. No correlation was observed in the case of 1298A/C polymorphism [64]. In contrast, the 677C/T polymorphism was not associated with the clinical response of metastatic colorectal cancer patients who received 5-FU/oxaliplatin/leucovorin chemotherapy and caused severe grade 3 to 4 diarrhoea [65]. Although the variant 677C/T did not affect the risk of colorectal cancer, it affected the sensitivity to chemotherapy and the risk of side-effects and therefore survival in stage III and possibly stage IV colon cancer [66]. It was also reported that patients initially treated with 5-FU had a severe toxicity when they were homozygous carriers of 1298C/C [67].

As pointed by out several studies [68–70] and documented by the above-described data, SNPs in the MTHFR gene cannot be considered as independent factors of outcome in colorectal cancer patients treated with 5-FU-based chemotherapy.

Polymorphisms of glutathione S-transferase

Glutathione S-transferases (GSTs) are members of a superfamily of dimeric phase II detoxifying enzymes. They protect cellular macromolecules from damage by catalyzing the conjugation of toxic and carcinogenic electrophilic molecules with glutathione. The resulting complex is less toxic and more readily excreted. In contrast to the other members of the family, such as GSTT1, GSTM1 and GSTA1, the variant GSTP1 is predominantly expressed in colon. Diverse common SNPs have been described for the GST genes that either abolish or decrease or increase the enzyme activity of GSTs for electrophilic substances [67].

To predict the toxicity in metastatic colorectal cancer patients treated with 5-FU/irinotecan/leucovorin regimens, the roles of 69C/T in GSTA1, 105Ile/Val (isoleucine/valine substitution) in GSTP1, and null allele (homozygous DNA deletion) in GSTT1 and GSTM1 were evaluated. In human tissues a decline in GSTP1 activity was reported to parallel with an increasing number of 105Ile/Val alleles in GSTP1 [71]. Thus, a valine residue at position 105 of the GSTP1 protein resulted in decreased enzyme activity. A strongly significant association was observed between the null allele in GSTT1 and gastrointestinal toxicity. In comparison to 23% of patients with an intact GSTT1 genotype, 57% of null allele carriers developed gastrointestinal grade 3 toxicity. Thus, patients with a null allele in GSTT1 had a greater probability of developing toxicity to 5-FU/irinotecan/leucovorin than patients who lacked this deletion. These findings indicated that the null allele in GSTT1 gene might be considered as a new marker when the treatment schedule is administered to metastatic colorectal cancer patients. Besides, the other polymorphisms analysed in that study did not refer to toxicity [72]. In addition, the neurotoxicity of patients with advanced colorectal cancer treated with a chemotherapy of 5-FU, oxaliplatin and folinic acid was reported to be significantly associated with the 105Ile/Val polymorphism in GSTP1. Carriers of the 105Val/Val genotype were more prone to suffer from grade 3 neurotoxicity than carriers of 105Ile/Val and 105Ile/Ile genotypes [67].

To predict the clinical outcome of 5-FU/oxaliplatin-based chemotherapy in patients with advanced colorectal cancer and refractory stage IV disease, the SNP 105Ile/Val in GSTP1 was examined. Stoehlmacher et al. demonstrated that the 105Ile/Val variant might be a predictor for time to progression to 5-FU/oxaliplatin, as well as overall survival. They suggested that patients with this variant allele may experience protracted detoxification of oxaliplatin, and that a prolonged exposure of the tumour to oxaliplatin may lead to an increased overall efficacy of the drug and a superior survival of the patients. In contrast, the null alleles of GSTT1 and the GSTM1 linked to an abolished enzyme activity had no effect on clinical outcome [73]. Moreover, the same laboratory showed that the 105Ile/Val polymorphism in GSTP1 was associated in a dose-dependent fashion with increased survival of patients with advanced

colorectal cancer receiving 5-FU/oxaliplatin chemotherapy. Patients with the genotypes 105Val/Val, 105Ile/Ile and 105Ile/Val had a median survival of 24.9, 7.9 and 13.3 months, respectively [74].

Although the published data are contradictory, they underline the fact that GST genotyping of individual colorectal cancer patients receiving 5-FU-based chemotherapy may contribute to improved therapy planning.

Interactions between the different genotypes

Some studies tried to go beyond the analyses of single genetic polymorphisms to a more comprehensive investigation that identifies genomic variants and patterns of several polymorphisms. However, also these investigations showed contradictory data, thus leading to increasing uncertainty in the identification of reliable predictive genetic markers of drug response. The interactions between ten candidate polymorphisms of nine genes and the incremental toxicity of adding irinotecan or oxaliplatin to FU were investigated in a large randomized trial. Among the different putative pharmacogenetic markers assessed, the polymorphisms of TS, DPD, MTHFR and GSTP1 were also analyzed. However, none of the polymorphisms was significantly associated with the toxicity of any regimen and the primary outcome [75]. Moreover, six gene polymorphisms, among others of GSTP1, MTHFR and TS, were assessed in Taiwanese patients who were diagnosed at high-risk stages and administered adjuvant chemotherapy regimens that included 5-FU and leucovorin. Also in this study, no correlations between genetic polymorphisms and clinicopathological features of the patients were detected [76]. However, Capitain et al. who investigated different germinal polymorphisms and 5-FU plasma clearance in patients of advanced colorectal cancer initially treated by 5-FU reported that the overall survival of the patients with a 3R/3R genotype of TS associated with the homozygosity of C/C for 677C/T or A/A for 1298A/C of MTHFR was statistically shorter [53].

These data show that there is a considerable need to increase efforts in the screening of several polymorphic variants in the 5-FU metabolic pathway to improve clinical outcome in the treatment of colorectal cancer.

Conclusions

The pharmacogenetics of drug-metabolizing systems deals with the identification of genetic polymorphisms that are responsible for inter-individual variation in the response to drug therapy. In the current chapter, pharmacogenetic studies investigating the drug targets of the 5-FU metabolism pathway in the treatment of colorectal cancers have been discussed. Several enzymes are involved in the catabolism and anabolism of 5-FU, and their activity varies largely between individuals. As a result the anti-tumour activity of 5-FU is limited by the genetic polymorphisms of these enzymes.

Inconsistent data on the relationship of the diverse variants of the thymidylate synthase, dihydropyrimidine dehydrogenase, methylene tetrahydrofolate reductase, orotate phosphoribosyltransferase and glutathione S-transferase to a better clinical outcome and survival of the patients, and to response to drug toxicity have been published. To date, this discrepancy underscores the complexity of the 5-FU metabolic pathway. Since several enzymes with diverse polymorphisms are involved in this pathway, it is questionable to only examine the polymorphisms of one enzyme and to ignore the impact of the genotypes of the other enzymes on the clinical outcome of the patients. In addition, the contradictory data amongst the various studies may be due to retrospective analyses and relatively small patient cohorts. Further reasons may be the performance of different techniques, such as immunohistochemistry and RT-PCR, which have been used to examine the expression of these enzymes, the genotyping in normal or tumour tissues, missing PCR-based microsatellite analyses for the evaluation of LOH, variable doses and schedules of 5-FU-based chemotherapy and variable tumour stages of the patients.

To enhance therapeutic effect and to minimize toxicity of 5-FU-based chemotherapies, it is important to gain a better understanding of the metabolism of 5-FU agents and to scrutinize which molecular determinants exert a dominating influence on the response to 5-FU. To advance 5-FU-based chemotherapies, multivariable approaches on diverse molecular markers and prospective clinical trials with large patient cohorts displaying statistical power are required. The identification of relevant germ-line polymorphisms as well as tumour-specific somatic mutations, combined with their mRNA or protein expression levels in tumour tissue, might permit more effective and individualised 5-FU-based chemotherapy in future. Selective genotypical and phenotypical information should facilitate the performance of individualised chemotherapy regimen and combinations of chemotherapeutic agents with the adequate doses of drugs. Technologies, such as microarray and proteomics, may lead to the identification of predictive markers and should improve the further development of patient treatment. Finally, for improving the outlook of patients with colorectal cancer, a number of new agents (e.g., capecitabine eniluracil, tomudex) targeted to the enzymes in 5-FU pathway have been synthesized, and novel combinations of 5-FU or its analogs with agents (e.g., oxaliplatin, irinotecan) have been examined.

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