Liver-only metastatic colorectal cancer patients and thymidylate synthase polymorphisms for predicting response to 5-fluorouracil-based chemotherapy

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We investigated the association between thymidylate synthase (TS) germline polymorphisms and response to 5-fluorouracil-based chemotherapy in 80 patients with liver-only metastatic colorectal cancer (MCRC). The tandem repeat polymorphism (VNTR) in TS 5'-untranslated region (5'-UTR), which consists of two (2R) or three (3R) 28-bp repeated sequences, with or without a G/C nucleotide change in 3R carriers (3G or 3C) and a 6-bp insertion/deletion (6+/6-) in the TS 3'-UTR, was studied. The distinction between high (2R/3G, 3C/3G and 3G/3G) and low (2R/2R, 2R/3C and 3C/3C) TS expression genotypes according to the 5'-UTR VNTR + G/C nucleotide change showed significant association with tumour response (P = 0.01). In particular, high TS expression genotypes were found in 8 out of 34 patients (23.5%) with complete or partial response and in 24 out of 46 patients (52%) with stable disease and disease progression. Liver-only MCRC patients are a homogeneous and clinically relevant subgroup that may represent an ideal setting for studying the actual influence of TS polymorphisms.

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Functional polymorphisms in the 5'-untranslated region (5'-UTR) and the 3'-UTR of the thymidylate synthase (TS) gene have been identified in the last decade (Marsh, 2005). A variable number of 28-bp tandem repeated sequence (VNTR) in TS 5'-UTR determines two (2R) or three (3R) alleles (Horie et al, 1995) and three common genotypes (2R/2R, 2R/3R and 3R/3R). Upregulated TS protein levels were found to be associated with the 3R allele (Kawakami et al, 1999; Yu et al, 2008). A G/C single-nucleotide polymorphism (SNP) in the 3R allele was found to determine additional alleles (3G or 3C) at this locus (Kawakami and Watanabe, 2003; Mandola et al, 2003), and according to their functional role, it allows a distinction between high (2R/3G, 3C/3G and 3G/3G) and low (2R/2R, 2R/3C and 3C/3C) TS expression genotypes in vivo (Morganti et al, 2005; Yawata et al, 2005). A more recently discovered TS genetic variant is a 6-bp insertion/deletion (6+/6-) in 3'-UTR (Ulrich et al, 2000). TS 3'-UTR genotypes (6+ 6+/6-- 6+ 6-- and 6--/6--) seem to be associated with variable TS mRNA levels (Mandola et al, 2004); however, the functional effect of the 3'-UTR polymorphism is not well defined yet (Calascibetta et al, 2004).

5-Fluorouracil is a fundamental drug in the treatment of patients with colorectal cancer, and TS levels are considered an important factor for explaining the differences in 5-fluorouracil antitumour activity (Popat et al, 2004). Therefore, the TS functional polymorphisms are under investigation for the possibility of optimising chemotherapy (Yong and Innocenti, 2007). Studies in patients with metastatic colorectal cancer showed that carriers of the TS 5'-UTR 3R (3G) and/or the TS 3'-UTR 6+ alleles had adverse clinical outcomes (Pullarkat et al, 2001; Etienne et al, 2002; Park et al, 2002; Marcuello et al, 2004; Stoehlmacher et al, 2004; Martinez-Balibrea et al, 2007); however, such an association was not always detected (Lecomte et al, 2004; Jakobsen et al, 2005; Ruzzo et al, 2007a, b). Heterogeneity in clinical experimental conditions (Sorbye et al, 2007), in tumour burden (Köhne et al, 2002) and in genetic/molecular features in the presence of a multisite metastatic disease (Yokota, 2000) may explain variable results in these pharmacogenetic studies.

We hypothesised that the 20–30% of patients with liver-only metastatic colorectal cancer (MCRC) (Mandalà et al, 2007) may represent a favourably homogeneous and clinically relevant setting for evaluating the role of TS polymorphisms for predicting response to 5-fluorouracil-based chemotherapy. For this purpose, we performed an analysis of TS polymorphisms in patients with liver-only MCRC who were previously enrolled in two prospective pharmacogenetic studies including 312 patients treated with first-line FOLFOX (bolus/infusional 5-fluorouracil coupled with
oxaliplatin) or FOLFIRI (bolus/infusional 5-fluorouracil coupled with irinotecan) regimens (Ruzzo et al, 2007a,b). FOLFOPX and FOLFIRI regimens are equally active and they produce comparable response rates in first-line chemotherapy. In both the regimens, 5-fluorouracil is used at the same dose and schedule (Colucci et al, 2005). The primary end point of the study was the association between TS polymorphism and tumour response.

MATERIALS AND METHODS

Study population

Three hundred and twelve patients with metastatic colorectal cancer were prospectively enrolled in two previous pharmacogenetic studies (Ruzzo et al, 2007a,b) and they underwent first-line chemotherapy including leucovorin 100 mg m\(^{-2}\) administered as a 2-h infusion before 5-fluorouracil 400 mg m\(^{-2}\) administered as an intravenous bolus injection and 5-fluorouracil 600 mg m\(^{-2}\) as a 22-h infusion immediately after FU bolus injection on days 1 and 2, every 2 weeks. Eighty patients (25.6%) had liver-only metastatic disease and they were included in this analysis. Ten of the 80 patients had history of liver surgery for metastasectomy and they were with liver-only relapse.

The 80 studied patients had cytologically or histologically confirmed metastatic colorectal cancer and the presence of at least one measurable lesion. Pretreatment evaluation included a complete medical and clinical–physical examination, KPS evaluation, baseline measurement of tumour size based on CT scan, serum chemistries and CEA. Objective response was evaluated after four cycles of treatment and then every 2 months according to the RECIST criteria (Therasse et al, 2000). For the purpose of this study, radiology studies of the 80 patients were reviewed for confirming the treatment outcomes. Patients’ characteristics and their outcomes were unknown to investigators performing genetic analyses. The study was approved by local ethical committees and patients provided signed informed consent.

Analysis of polymorphisms

A blood sample from each enrolled patient was used for genotyping and it was collected before starting chemotherapy. Genomic DNA was extracted from 200 µl whole blood using the QiaAmp kit (Qiagen, Valencia, CA, USA). All polymorphisms were investigated using a PCR-restriction fragment length polymorphism technique. The assays for studying polymorphisms were performed as described previously (Ruzzo et al, 2007a,b).

Statistical analyses

The primary end point of the study was the association between TS polymorphisms in patients with liver-only MCRC and response to 5-fluorouracil-based chemotherapy. Genotype frequencies were checked for agreement with those expected under the Hardy–Weinberg equilibrium. Genotypes for each polymorphism were investigated using a PCR-restriction fragment length polymorphism technique. The assays for studying polymorphisms were performed as described previously (Ruzzo et al, 2007a,b).

Complete LD and \(r^2 = 0\) indicating absent LD. Haplotype frequencies were reconstructed in the study population of responders and non-responders. Association of haplotypes with clinical outcome was estimated by comparing haplotype distributions among dichotomised patients using the \(\chi^2\)-test.

RESULTS

The characteristics of the 80 studied patients and the overall frequencies of genotypes are shown in Table 1. All patients were assessable for response and they received a minimum of four cycles of chemotherapy. In the 80 patients, there was one complete response (1.2%), 33 partial responses (41.2%), 30 stable diseases (37.6%) and 16 progressions (20%). Median age was 63 years (minimum 38 years and maximum 75 years). Liver metastases were synchronous in 22 patients (27.5%) and metachronous in 68 patients (72.5%). The frequencies of genotypes are in Hardy–Weinberg equilibrium and they are consistent with those observed in Caucasian ethnicity (Archive of Genetic Association Studies accessible at: http://geneticsassociationdb.nih.gov/).

No significant association between clinicopathological features and tumour response was found (Table 2). The analysis of the three polymorphisms and response is shown in Table 3. The Bonferroni-adjusted \(P\)-value for the three comparisons is 0.05/3, \(P = 0.016\). The TS \(3^\prime\)-UTR VNTR with G/C polymorphism in 3R alleles showed association with treatment outcome (\(P = 0.011\)). In particular, high TS expression genotypes (2R/3G, 3C/3G and 3C/3R) were associated with complete or partial response (patients with stable disease or response progression). The \(\chi^2\)-test was used for comparing proportions. Statistical significance was defined as \(P < 0.05\). A Bonferroni correction of the \(P\)-value for multiple comparisons was used where applicable.

The SHEsis software platform (http://202.120.7.14/analysis/myAnalysis.php) was used to estimate haplotype frequencies and the presence of linkage disequilibrium (LD). Linkage disequilibrium exists between two SNPs, if their variants appear together more often than expected (non-random inheritance). Linkage disequilibrium was estimated using \(r^2\), with \(r^2 = 1\) indicating

| Table 1 | Characteristics of the 80 patients and genotype frequencies |
|---------|----------------------------------------------------------|
| Sex     | Male 48 (60) Female 32 (40) |
| Karnofsky performance status | 90–100 58 (72) 80 22 (28) |
| Resected primary tumour | Yes 70 (87) No 10 (13) |
| Prior adjuvant therapy | None 44 (55) Yes 36 (45) |
| Carcinoe embryonic antigen | \(\leq 10\) ng ml\(^{-1}\) 56 (70) \(> 10\) ng ml\(^{-1}\) 24 (30) |
| Genotypes | TS \(3^\prime\)-UTR VNTR\(^3\) |
| 2R/2R | 16 (20) |
| 2R/3R | 38 (47) |
| 3R/3R | 26 (33) |
| TS \(3^\prime\)-UTR VNTR+G/C\(^3\) | 2R/2R, 2R/3C, 3C/3C |
| 2R/3G, 3C/3G, 3G/3G | 48 (60) |
| 3G/3G | 32 (40) |
| TS \(3^\prime\)-UTR | −6/−6 30 (38) −6/+6 40 (50) +6/+6 10 (12) |

\(^3\) The variable number of tandem repeats (VNTR) polymorphism is a two (2R) or three (3R) 28-bp tandem repeat sequence in TS \(3^\prime\)-UTR. A single-nucleotide change in 3R allele is a second polymorphism that distinguishes 3G carriers (2R/3G, 3G/3G and 3G/3C genotypes) from non-3G carriers (2R/2R, 2R/3C and 3C/3C genotypes).
**Table 2** Characteristics of the patients and tumour response

| Characteristics                        | No. of patients (%) | Responders* (N = 34) | Non-responders* (N = 46) | P-value |
|----------------------------------------|---------------------|-----------------------|--------------------------|---------|
| Karnofsky performance status           |                     |                       |                          |         |
| 90–100                                 | 58 (72)             | 26 (76)               | 32 (70)                  | 0.6     |
| 80                                     | 22 (28)             | 8 (24)                | 14 (30)                  |         |
| Resected primary tumour                |                     |                       |                          |         |
| Yes                                    | 67 (84)             | 30 (88)               | 37 (80)                  | 0.5     |
| No                                     | 13 (16)             | 4 (12)                | 9 (20)                   |         |
| Prior adjuvant therapy                 |                     |                       |                          |         |
| None                                   | 44 (55)             | 21 (62)               | 23 (50)                  | 0.4     |
| Yes                                    | 36 (45)             | 13 (38)               | 23 (50)                  |         |
| Carcinoembryonic antigen               |                     |                       |                          |         |
| ≤10 ng ml⁻¹                            | 56 (70)             | 26 (76)               | 30 (65)                  | 0.4     |
| >10 ng ml⁻¹                            | 24 (30)             | 8 (24)                | 16 (35)                  |         |

*Responders are patients with complete or partial response. Non-responders are patients with stable disease or disease progression.

**Table 4** Association between genotypes and response to chemotherapy in additive and recessive models

| Genotypes     | No. of patients (%) | Responders* (N = 34) | Non-responders* (N = 46) | P-value |
|---------------|---------------------|-----------------------|--------------------------|---------|
| TS 5′-UTR     |                     |                       |                          |         |
| 2R/2R         | 16 (20)             | 10 (30)               | 6 (13)                   | 0.19    |
| 2R/3R         | 38 (47)             | 14 (40)               | 24 (52)                  |         |
| 3R/3R         | 26 (33)             | 10 (30)               | 16 (35)                  |         |
| TS 5′-UTR²    |                     |                       |                          |         |
| 2R/2R, 2R/3R | 48 (60)             | 26 (76)               | 22 (48)                  | 0.011¹  |
| 3G/3G/3G     | 2R/3G, 3G/3C, 3G/3G| 32 (40)               | 8 (24)                   |         |
| TS 3′-UTR     |                     |                       |                          |         |
| −6/−6         | 30 (38)             | 11 (32)               | 19 (41)                  | 0.37    |
| −6/+6         | 40 (50)             | 20 (59)               | 20 (44)                  |         |
| +6/+6         | 12 (12)             | 3 (9)                 | 7 (15)                   |         |

*Responders are patients with complete or partial response. Non-responders are patients with stable disease or disease progression.

3G/3G) were found in 8 out of 34 patients with complete or partial response (23.5%) and 24 out of 46 patients with stable disease and disease progression (52%). The 5′-UTR VNTR and the 3′-UTR 6-bp insertion/deletion (+6−6) did not show association with tumour response. To further evaluate these two variants, their distribution was explored in recessive and additive models also, but without finding significant associations (Table 4).

The TS 5′-UTR and TS 3′-UTR loci showed mild LD (r² = 0.17). As shown in Table 5, non-3G haplotypes were prevalent in responders and 3G haplotypes in non-responders, with significantly different distribution of the 3G/6− haplotype.

Eleven responsive patients underwent liver surgery for resection of the residual metastatic disease (13.7%). Clear resection margins with removal of all known metastatic lesions were attained in these patients, with 10 of them carrying one of the low TS expression genotypes (2R/2R, 2R/3C and 3C/3C). At the time of data analysis (March 2008), 78 patients suffered from disease progression (97.5%). For addressing an exploratory analysis of time to progression in patients with high and low TS expression genotypes, time to event distributions were studied using the Kaplan–Meier method. As shown in Figure 1, the results support the influence of the TS 5′-UTR VNTR with G/C SNP on the outcome of these patients.

**DISCUSSION**

To the best of our knowledge, this is the first analysis of TS polymorphisms in patients with liver-only MCRC. In comparison with previous studies (Pullarkat et al, 2001; Etienne et al, 2002;
The findings suggest that the double assessment of the VNTR plus G/C nucleotide change with dichotomisation of patients into carriers of high (2R/3G, 3C/3G and 3G/3G) and low (2R/2R, 2R/3C and 3C/3C) TS expression genotypes may not suffer from the possible presence of LOH. The finding of the association between TS 5′-UTR VNTR + G/C and tumour response may not only reflect a better functional characterisation of 3C and 3G alleles, but also a less extensive influence of tumour LOH on the germline assessment for high-TS expression genotypes (2R/3G, 3C/3G and 3G/3G).

In conclusion, the homogeneous subgroup of patients with metastatic colorectal cancer (Köhne et al., 2002), and in our opinion, TS polymorphisms deserved the present investigation more than other genetic variants with putative influence on 5-fluorouracil activity (i.e. methylenetetrahydrofolate reductase gene polymorphisms). In addition to the fact that TS is the target enzyme of 5-fluorouracil, it has been observed that TS levels may be dynamic, with upregulation after fluoropyrimidine exposure (Uchida et al., 2004a, b; Mauritz et al., 2007). In particular, this effect was described in liver metastases from colorectal cancer in patients who received bolus 5-fluorouracil (Mauritz et al., 2007). Therefore, TS polymorphisms may influence the outcome to 5-fluorouracil chemotherapy, not only for their role in determining different baseline levels of TS activity (Marsh, 2003), but also for modulating the enhancement of TS levels in response to 5-fluorouracil. In fact, the 5-fluorouracil-induced upregulation of TS mRNA may be greater in carriers of high-expression TS genotypes than in carriers of low-expression TS genotypes. We cannot rule out, however, that a double assessment of TS and methylenetetrahydrofolate reductase polymorphisms may improve the predictive role of the single analysis of TS polymorphisms.

Another reason for studying pharmacogenetics in liver-only MCRC patients is related to the lack of predictive factor for response to neoadjuvant chemotherapy. Liver surgery can provide long-term survival for liver-only, metastatic colorectal cancer patients, but liver metastasectomy is feasible in only 15–25% of the patients. Neoadjuvant chemotherapy can provide response rates as high as 50%, allowing liver metastasectomy in about 10–15% of patients initially deemed unresectable. Tumour response to preoperative chemotherapy seems to be associated with outcome following liver resection for colorectal metastases (Folprecht et al., 2005) and, if genetically predictable, it could be improved by the selective choice of available drugs. In this study, 10 of the 11 responsive patients who underwent liver surgery were carriers of low TS expression genotypes (2R/2R, 2R/3C and 3C/3C). Actually, we performed an analysis of TS polymorphisms for response to 5-fluorouracil and we did not address this study to pharmacogenetics for liver metastases resectability and survival after preoperative chemotherapy. These end points require a prospective study, including a baseline multidisciplinary evaluation of the unresectable liver disease and long-term follow-up.

In conclusion, the homogeneous subgroup of patients with liver-only metastatic disease allowed the predictive role of TS polymorphisms to stand out. In fact, the association between polymorphisms and tumour response was included in the secondary end points of our two previous pharmacogenetic studies (Ruzzo et al., 2007a, b), but these analyses failed to demonstrate a predictive role for the genetic variants. The clinical setting assumes a relevant role for exploring the pharmacogenetic associations in patients with metastatic cancer and additional studies are warranted for confirming our findings.

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REFERENCES

Calascibetta A, Cabibi D, Martorana A, Sanguedolce G, Rausa I, Leo S, Dardanoni G, Sanguedolce R (2004) Thymidylate synthase gene promoter polymorphisms are associated with TmRNA expressions but not with microsatellite instability in colorectal cancer. Anticancer Res 24: 3875–3880

Colucci G, Gobbia V, Paoletti G, Giuliani F, Caruso M, Gobbia N, Carteni G, Agostara B, Pezzella G, Manzione L, Borsellino N, Misina A, Romito M, Durini E, Cordio S, Di Seri M, Lopez M, Maieil E, Montemuro S, Cramerossa A, Lorusso V, Di Biseglie M, Chiarezza M, Valerio MR, Guida T, Leonardi V, Picsoni S, Rosati G, Carrozza F, Nettis G, Valsesi M, Filippelli G, Fortunato S, Mancarella S, Brunetti G, Gruppo Oncologico Dell’Italia Meridionale (2005) Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell’Italia Meridionale. J Clin Oncol 23: 4866–4875

Etienne MC, Chazal M, Laurent-Puig P, Magne N, Rosty C, Formento JL, Francoual M, Formento P, Renée N, Chamorey E, Bourgeon A, Seitz JF, Delpero JR, Letoublon C, Pezet D, Milano G (2002) Prognostic value of tumoral thymidylate synthase and p53 in metastatic colorectal cancer patients receiving fluorouracil-based chemotherapy: phenotypic and genotypic analyses. J Clin Oncol 20: 2832–2843

Polprecht G, Grothey A, Alberts S, Raab HR, Köhne CH (2005) Neoadjuvant treatment of unresectable colorectal liver metastases: correlation between tumour response and resection rates. Ann Oncol 16: 1311–1319

Horie N, Aiba H, Oguro K, Hojo H, Takeishi K (1995) Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5’-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct Funct 20: 191–197

Hoshino S, Yamashita Y, Maekawa T, Shirakusa T (2005) Effects on DNA and RNA after the administration of two different schedules of 5-fluorouracil in colorectal cancer patients. Cancer Chemother Pharmacol 56: 648–652

Jakobsen A, Nielsen JN, Gyldenkærne N, Lindeberg J (2005) Thymidylate synthase and methylene tetrahydrofolate reductase gene polymorphism in normal tissue as predictors of fluorouracil sensitivity. J Clin Oncol 23: 1365–1369

Kawakami K, Ishida Y, Danenberg KD, Omura K, Watanabe G, Danenberg PV (2002) Functional polymorphism of the thymidylate synthase gene in colorectal cancer accompanied by frequent loss of heterozygosity. Jpn J Cancer Res 93: 1221–1229

Kawakami K, Omura K, Kamehira E, Watanabe Y (1999) Polymorphic tandem repeats in the thymidylate synthase gene is associated with its protein expression in human gastrointestinal cancers. Anticancer Res 19: 3249–3252

Kawakami K, Watanabe G (2003) Identification and functional analysis of single nucleotide polymorphism in the tandem repeat sequence of the thymidylate synthase gene. Cancer Res 63: 6004–6007

Köhne CH, Cunningham D, Di CF, Glimelius B, Blijham G, Aranda E, Kawakami K, Ishida Y, Danenberg KD, Omura K, Watanabe G, Danenberg PV (2004a) Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer. Br J Cancer 90: 438–444

Mandola MV, Stoehlmacher J, Zhang W, Troen-Wei D, Groshen S, Lenz HJ (2002) Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int J Colorectal Dis 17: 46–49

Popat S, Matakidou A (2001) Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. J Clin Oncol 22: 529–536

Ruzzo A, Graziano F, Loupakis F, Rulli E, Canestri E, Santini D, Catalano V, Ficarelli R, Maltese P, Bionnisi M, Ghiavoni G, Giordani P, Giustini L, Falcone A, Tonini G, Silva R, Mattioli R, Floriani I, Magnani M (2007a) Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX-4 chemotherapy. J Clin Oncol 25: 1247–1254

Ruzzo A, Graziano F, Loupakis F, Santini D, Catalano V, Bionnisi M, Ficarelli R, Fontana A, Andreoni F, Falcone A, Canestri E, Tonini G, Mari D, Lippe P, Pizzagalli F, Schiavon G, Alessandroni P, Giustini L, Maltese P, Testa E, Menichetti ET, Magnani M (2007b) Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFIRI chemotherapy. Pharmacogenomics 8(4): 278–288

Sorbye H, Köhne CH, Sargent DJ, Giulianelli B (2007) Patient characteristics and stratification in medical treatment studies for metastatic colorectal cancer: a proposal for standardization of patient characteristic reporting and stratification. Ann Oncol 18: 1666–1672

Stoehlmacher J, Park DJ, Zhang W, Yang D, Groshen S, Zahedy S, Lenz HJ (2006) Multivariate analysis of genomic polymorphisms: prediction of clinical outcome to 5-FU/oxaliplatin combination chemotherapy in refractory colorectal cancer. Br J Cancer 91: 344–354

Therasse P, Arnbck SG, Eisenhaauer EA, Wanders J, Kaplan RS, Rubinstein L, Yervjei V, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92: 205–216

Trillet-Lenoir V, Freyer G, Kaemmerlen P, Fond A, Pellet O, Lombard-Bohas C, Gaulin JL, Lledo G, Mackiewicz R, Goutelbe MC, Moindrot H, Boyer JD, Chassagnel L, Stremdsoer N, Desseigne F, Moreau JM, Hulcielus F, Moraillon A, Chapius F, Blesse JP, Barbier Y, Dittmann MO, Valette P (2002) Assessment of tumor response to chemotherapy for metastatic colorectal cancer: accuracy of the RECIST criteria. Br J Radiol 75: 903–908

Uchida K, Hayashi K, Kawakami K, Schneider S, Tochin JM, Kuramochi H, Takasaki K, Danenberg KD, Danenberg PV (2004a) Loss of heterozygosity at the thymidylate synthase (TS) locus on chromosome 18 affects tumor response and survival in individuals heterogeneous for a 28-bp polymorphism in the TS gene. Cancer Res 64: 1916–1923

Uchida K, Hayashi K, Kuramochi H, Takasaki K (2004b) Changes in intratumoral thymidylate synthase (TS) and dihydroxyprimidine dehydrogenase (DPD) mRNA expression in colorectal and gastric cancer during continuous tefarquif infusion. Int J Oncol 19: 341–346

Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FW, Potter JD (2000) Searching expressed sequence Tag databases: discovery and confirm-
tion of a common polymorphism in the thymidylate synthase gene.  
*Cancer Epidemiol Biomarkers Prev* **9**: 1381–1385

Yawata A, Kim SR, Miyajima A, Kubo T, Ishida S, Saito Y, Nakajima Y, Katori N, Matsumoto Y, Fukuoka M, Ohno Y, Ozawa S, Sawada J (2005) Polymorphic tandem repeat sequences of the thymidylate gene correlates with cellular-based sensitivity to fluoropyrimidine antitumor agents.  
*Cancer Chemother Pharmacol* **56**: 465–472

Yokota J (2000) Tumor progression and metastasis.  
*Carcinogenesis* **21**: 497–503

Yong WP, Innocenti F (2007) Translation of pharmacogenetic knowledge into cancer therapeutics.  
*Clin Adv Hematol Oncol* **5**: 698–706

Yu KH, Wang WX, Ding YM, Li H, Wang ZS (2008) Polymorphism of thymidylate synthase gene associated with its protein expression in human colon cancer.  
*World J Gastroenterol* **14**: 617–621