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Letter to the Editors

5-Fluorouracil-based chemotherapy for colorectal cancer and *MTHFR/MTRR* genotypes

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Treatment with 5-fluorouracil (5-FU) is known to improve survival in various cancers. The largest impact of the drug has been reported in colorectal cancer [1]. Active metabolites of 5-FU disrupt both DNA and RNA synthesis through a mechanism involving the folate metabolic pathway [2–4]. The overall response rate to 5-FU in advanced colorectal cancer is limited to 10–15%. Although survival is reported to improve after the addition of irinotecan and oxaliplatin to the 5-FU regimen, toxicity increases [5, 6]. Crucially, response to chemotherapeutics, overall survival (OS) and attendant toxicity is associated with large inter-individual variations [7]. Elevated concentrations of 5,10-methylenetetrahydrofolate (CH_2THF), which are critical for the optimal effect of 5-FU, are dependent on methylenetetrahydrofolate reductase (MTHFR), an enzyme that reduces it irreversibly to 5-methyltetrahydrofolate. A link between an improved response to 5-FU treatment and two common polymorphisms in the *MTHFR* gene, associated with reduced enzymatic activity, has been discerned through *in vitro* experiments. While an increased sensitivity to the drug was observed in colon and breast cancer cell lines transfected with the variant 677T *MTHFR* cDNA, 19 different cell lines with the 677 C > T and 1298 A > C variants displayed increased folate concentrations and increased 5-FU efficacy, respectively [8, 9]. The MTHFR activity in tissue is presumed to be a major determinant of clinical response but the pharmacogenetic studies on the effect of the two polymorphisms have not always been consistent [10]. A recent study carried out on 117 colorectal cancer patients showed specific linkage of response to FOLFOX therapy with the two *MTHFR* polymorphisms [11].

We investigated the role of six genetic variants in the *MTHFR* and *5-methylenetetrahydrofolate-homocysteine methyltransferase reductase* (*MTRR*) genes on the clinical outcome in 273 colorectal cancer patients who were administered a 5-FU based regimen as first-line post-operative therapy. The patients were treated by an intended curative surgery followed by a 5-FU based adjuvant therapy regimen. The adjuvant therapy consisted of either a Mayo regimen, delivered as a bolus infusion of 5-FU (425 mg m^{-2}) and leucovorin (10 mg m^{-2}) for 5 days every 4 weeks six times or a simplified DeGramond regimen which consisted of a 2 h intravenous (i.v.) infusion of leucovorin (200 mg m^{-2}), then a 5-FU i.v. bolus (400 mg m^{-2}) followed by a 46 h 5-FU continuous i.v. infusion (2400–3000 mg m^{-2}).

The patients were diagnosed between September 2004 and August 2009 in the Czech Republic, a country with one of the highest incidence rates, and belonged to a large cohort being investigated for the effect of genetic variability on the disease. The study design was approved by the Ethics Committee of the Institute of Experimental Medicine, Prague, Czech Republic. In this study the outcome variables measured were tumour stage, OS (time from operation till death or censorship) and progression-free survival (PFS, time from operation till progression, death or censorship). The genotyping was carried out using allelic discrimination method and validated by random re-genotyping and direct DNA sequencing. The median survival time of deceased patients was 21 months. Five established prognostic factors (pT, pN and pM, tumour grading and histological type) associated with survival were included as covariates in subsequent Cox regression analyses for assessing the relationship between the polymorphisms and clinical outcome. Patient characteristics like age at diagnosis, duration of therapy, family history and diabetes (self-reported) did not affect OS (probability values higher than 0.13). Out of six polymorphisms investigated, the variant 1298 A > C in *MTHFR* showed association with PFS. The patients with AC ($n = 102$) and CC

($n = 22$) genotypes showed an increased PFS with an adjusted hazard ratio (HR) of 0.52 (95% CI 0.30–0.89) compared with patients with the AA genotype ($n = 93$). None of the other investigated variants including the 66 A > G, 524 C > T, 1049 A > G and 1793 C > T polymorphisms in the *MTRR* and the 677 C > T in the *MTHFR* gene showed any association.

Clinical studies on genetic variation in the folate metabolism pathway and colorectal cancer survival have mainly focused on patients undergoing treatment for metastatic colorectal cancer and patients who were administered adjuvant therapy before or after surgery. Critically, most of the reported studies were conducted in a small number of patients (less than 150) and very often the treatment regime consisted of 5-FU and additional antineoplastic drugs. In addition to treatment homogeneity, this study, together with two earlier investigations, comprises one of the largest patient groups. While the study by Afzal *et al*. did not find any modulation in survival due to polymorphisms in patients treated with 5-FU; the study by Boige *et al*. showed a predictive effect due to the *MTHFR* 1298 A > C polymorphism in patients receiving oxaliplatin following LV5FU2 [2, 6]. Thus, data from our study coupled with those reported earlier, in particular the one by Etienne-Grimaldi *et al*. augment an argument for the further investigation of the role of variants in genes involved in folate metabolism, *MTHFR* in particular, on the effect of 5-FU based chemotherapeutics for treatment of colorectal cancer [11]. 5-FU represents an early success in the relentless endeavours directed towards treatment of cancers and has remained a mainstay treatment. Identification of markers for predicting individual response will be another step towards personalized medicine in the genomic era.

Competing Interests

There are no competing interests to declare.

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REFERENCES

1 Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003; 3: 330–8.

2 Afzal S, Jensen SA, Vainer B, Vogel U, Matsen JP, Sorensen JB, Andersen PK, Poulsen HE. MTHFR polymorphisms and 5-FU-based adjuvant chemotherapy in colorectal cancer. Ann Oncol 2009; 20: 1660–6.

3 Canman CE, Lawrence TS, Shewach DS, Tang HY, Maybaum J. Resistance to fluorodeoxyuridine-induced DNA damage and cytotoxicity correlates with an elevation of deoxyuridine triphosphatase activity and failure to accumulate deoxyuridine triphosphate. Cancer Res 1993; 53: 5219–24.

4 Canman CE, Tang HY, Normolle DP, Lawrence TS, Maybaum J. Variations in patterns of DNA damage induced in human colorectal tumor cells by 5-fluorodeoxyuridine: implications for mechanisms of resistance and cytotoxicity. Proc Natl Acad Sci USA 1992; 89: 10474–8.

5 de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 2000; 18: 2938–47.

6 Boige V, Mendiboure J, Pignon JP, Loriot MA, Castaing M, Barrois M, Malka D, Tregouet DA, Bouche O, Le Corre D, Miran I, Mulot C, Ducreux M, Beaune P, Laurent-Puig P. Pharmacogenetic assessment of toxicity and outcome in patients with metastatic colorectal cancer treated with LV5FU2, FOLFOX, and FOLFIRI: FFCD 2000–05. J Clin Oncol 2010; 28: 2556–64.

7 Ulrich CM, Robien K, McLeod HL. Cancer pharmacogenetics: polymorphisms, pathways and beyond. Nat Rev Cancer 2003; 3: 912–20.

8 Sohn KJ, Croxford R, Yates Z, Lucock M, Kim YI. Effect of the methylenetetrahydrofolate reductase C677T polymorphism on chemosensitivity of colon and breast cancer cells to 5-fluorouracil and methotrexate. J Natl Cancer Inst 2004; 96: 134–44.

9 Etienne MC, Ilc K, Formento JL, Laurent-Puig P, Formento P, Cheradame S, Fischel JL, Milano G. Thymidylate synthase and methylenetetrahydrofolate reductase gene polymorphisms: relationships with 5-fluorouracil sensitivity. Br J Cancer 2004; 90: 526–34.

10 Etienne-Grimaldi MC, Francoual M, Formento JL, Milano G. Methylenetetrahydrofolate reductase (MTHFR) variants and fluorouracil-based treatments in colorectal cancer. Pharmacogenomics 2007; 8: 1561–6.

11 Etienne-Grimaldi MC, Milano G, Maindrault-Goebel F, Chibaudel B, Formento JL, Francoual M, Lledo G, Andre T, Mabro M, Mineur L, Flesch M, Carola E, de Gramont A. Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and FOLFOX response in colorectal cancer patients. Br J Clin Pharmacol 2010; 69: 58–66.

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