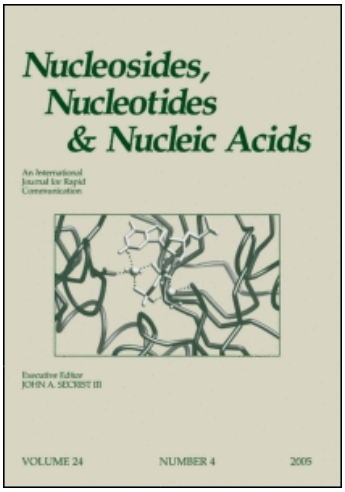

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Polymorphism of the Thymidylate Synthase Gene and Thymidylate Synthase Levels in Colon Cancer Cell Lines and Different Tissues of Colorectal Cancer Patients

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ABSTRACT

In a panel of 18 colon cancer cell lines we found that the thymidylate synthase (TS) genotype was related to TS enzyme activity, but not to TS protein and mRNA levels. In addition, no relation with drug sensitivity was observed. TS genotyping of different tissues from 78 colorectal cancer patients revealed a high level of homology in polymorphic status between normal and malignant tissues and the heterozygous genotype to be the most frequent.

Key Words: Thymidylate synthase; 5-Fluorouracil; Colorectal carcinoma; Polymorphism.

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INTRODUCTION

The enhancer region of the TS gene (TSER) contains a tandem repeat polymorphism which is thought to influence TS expression: the triple repeat (3R/3R) genotype might be associated with higher TS levels than the double repeat (2R/2R) or heterozygous (2R/3R) genotype.[1,2] Most studies thus far focused on the relation between TS genotype and survival of patients treated with 5-fluorouracil-based chemotherapy.[3–5] We analyzed the number of tandem repeats in a panel of selected and non-selected colon cancer cell lines and determined the relation with TS levels and sensitivity to TS inhibitors. Furthermore, we analyzed the TSER polymorphism in normal and malignant tissues of patients with advanced colorectal cancer.

MATERIALS AND METHODS

DNA was obtained from colon cancer cell lines (see Table 1) and normal and malignant tissues of patients with colorectal cancer. TSER genotype was assessed by PCR amplification as described by Horie et al.[1] and Marsh et al.[6] Drug sensitivity, TS mRNA expression and protein activity were measured as described previously.[7,8]

RESULTS AND DISCUSSION

Most of the 18 colon cancer cell lines showed the 2R/2R genotype (Table 1). Higher TS catalytic activity was observed in the cell lines with one or two triple repeat alleles, however FdUMP binding appeared to be lower (Table 2). No relation between TS genotype and TS protein, mRNA levels and sensitivity to TS inhibitors was observed in these cell lines.

In addition to the cell lines, samples of colon cancer patients are currently being studied. To date the TSER variant has been determined in different tissues of 78 patients. From 30 patients multiple tissue types were analyzed. Confirming a previous study we found a high level of homology of TSER genotypes between normal and malignant tissues from the same patient.[9] In one patient the TSER genotype of the

Table 1. TSER genotype of selected and unselected colon cancer cell lines.

Cell line	Genotype	Cell line	Genotype	Cell line	Genotype
SW620	2R/2R	Colo205	2R/2R	WiDr	2R/2R
SW1116	2R/2R	Colo201	2R/3R	WiDr-cPEM[b]	2R/2R
SW1398	2R/2R	Colo320	2R/2R	WiDr-4PEM[b]	2R/2R
SNU-C4	2R/2R	H630	3R/3R	WiDr/F[c]	2R/2R
SNU-C1	3R/3R	H630-R1[a]	3R/3R	Lovo	2R/2R
HT29	2R/2R	H630-R10[a]	3R/3R	LS174T	2R/3R

[a]5-FU resistant.
[b]Resistant to pemetrexed Sigmond. (From Ref. [9].)
[c]Grown under low folate conditions.

Table 2. Relation TSER genotype of unselected colon cancer cell lines with several parameters.

	TSER 2/3 + 3/3	TSER 2/2	*p*
TS activity (pmol/hr/mg protein)	5414	1705	0.03
FdUMP binding (pmol/mg protein)	664	1593	0.03
TS protein (pg)	385	669	0.60
TS mRNA (ratio β-actin)	10	7.2	0.60
Drug sensitivity (IC_{50}):			
5-FU (μM)	9.4	6.6	0.65
Tomudex (nM)	8.6	4.6	0.88
Pemetrexed (nM)	156	577	0.10

Sensitivity data and TS levels are partly from Van Triest. (From Ref. [7].)

primary tumor differed from that in liver metastasis and normal liver tissue. In the current group of patients the heterozygous TSER variant is the most common genotype (2R/3R: 37%; 2R/2R: 26%; 3R/3R: 28%). The TS genotypes will be compared to TS enzyme and mRNA expression levels in addition to survival data. Previous studies suggested that the triple repeat (3R/3R) genotype might be associated with higher TS protein levels than the 2R/2R or heterozygous genotype.[1,2] Since a high TS expression has been related to a poor response of colorectal cancer patients to treatment with 5-fluorouracil (5-FU), it has been anticipated that the TSER genotype is related to survival of colorectal cancer patients treated with 5-FU. Marsh et al. determined TSER genotypes of 121 patients with colorectal cancer and, in a subset of 24 patients with follow-up data, found that 3R/3R patients had a shorter survival than 2R/2R individuals.[10] Villafranca et al. observed that rectum carcinoma patients with the 3R/3R polymorphism had a lower change of downstaging after preoperative 5-FU–based chemoradiation than patients with 2R/3R or 2R/2R.[3] A significant relation between TSER genotype and response to 5-FU–based treatments was observed in two studies by the group of Lenz.[4,5] Prospective studies are warranted to determine the predictive value of TS genotyping.

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REFERENCES

1. Horie, N.; Aiba, H.; Oguro, K.; Hojo, H.; Takeishi, K. 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. **1995**, *20*, 191–197.
2. Kawakami, K.; Salonga, D.; Park, J.M.; Danenberg, K.D.; Uetake, H.; Brabender, J.; Omura, K.; Watanabe, G.; Danenberg, P.V. Different lengths of a polymorphic

repeat sequence in the thymidylate synthase gene affect translational efficiency but not its gene expression. Clin. Cancer Res. **2001**, *7*, 4096–4101.
3. Villafranca, E.; Okruzhnov, Y.; Dominguez, M.A.; Garcia-Foncillas, J.; Azinovic, I.; Martinez, E.; Illarramendi, J.J.; Arias, F.; Martinez, M.R.; Salgado, E.; Angeletti, S.; Brugarolas, A. Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. J. Clin. Oncol. **2001**, *19*, 1779–1786.
4. Pullarkat, S.T.; Stoehlmacher, J.; Ghaderi, V.; Xiong, Y.P.; Ingles, S.A.; Sherrod, A.; Warren, R.; Tsao-Wei, D.; Groshen, S.; Lenz, H.J. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. Pharmacogenomics J. **2001**, *1*, 65–70.
5. Park, D.J.; Stoehlmacher, J.; Zhang, W.; Tsao-Wei, D.; Groshen, S.; Lenz, H.J. Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int. J. Colorectal Dis. **2002**, *17*, 46–49.
6. Marsh, S.; Collie-Duguid, E.S.; Li, T.; Liu, X.; McLeod, H.L. Ethnic variation in the thymidylate synthase enhancer region polymorphism among Caucasian and Asian populations. Genomics **1999**, *58*, 310–312.
7. Van Triest, B.; Pinedo, H.M.; Van Hensbergen, Y.; Smid, K.; Telleman, F.; Schoenmakers, P.S.; Van der Wilt, C.L.; Van Laar, J.A.; Noordhuis, P.; Jansen, G.; Peters, G.J. Thymidylate synthase level as the main predictive parameter for sensitivity to 5-fluorouracil, but not for folate-based thymidylate synthase inhibitors, in 13 nonselected colon cancer cell lines. Clin. Cancer Res. **1999**, *5*, 643–654.
8. Rots, M.G.; Willey, J.C.; Jansen, G.; Van Zantwijk, C.H.; Noordhuis, P.; DeMuth, J.P.; Kuiper, E.; Veerman, A.J.; Pieters, R.; Peters, G.J. mRNA expression levels of methotrexate resistance-related proteins in childhood leukemia as determined by a standardized competitive template-based RT-PCR method. Leukemia **2000**, *14*, 2166–2175.
9. Sigmond, J.; Backus, H.H.J.; Wouters, D.; Temmink, O.H.; Jansen, G.; Peters, G.J. Induction of resistance to the multitargeted antifolate Pemetrexed (ALIMTA) in WiDr human colon cancer cells is associated with thymidylate synthase overexpression. Biochem. Pharmacol. **2003**, *66*, 431–438.
10. Marsh, S.; McKay, J.A.; Cassidy, J.; McLeod, H.L. Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. Int. J. Oncol. **2001**, *19*, 383–386.