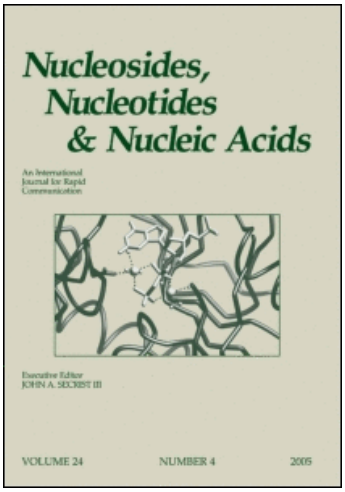

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Thymidylate Synthase Polymorphism and Microsatellite Instability: Association in Colorectal Cancer

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ABSTRACT

5-Fluorouracil (5FU) is the main drug used for the treatment of colorectal cancer (CRC) and Thymidilate Synthase (TS) is its target enzyme. TS gene has regulatory tandemly repeated sequences in its 5′ and 3′untraslated region (5′–3′ UTR). CRC often shows a kind of genomic instability called Microsatellite Instability (MSI) that is associated with TS levels and survival. Our data show that the genotype 2R/2R (homozygosity for 2 tandem repeat sequences in the 5′UTR) is more frequently associated with MSI+ and lower TS levels. More over we did not find any significant association between the 2R/3R (heterozygosity for 2 and 3 tandem repeat sequences in the 5′UTR) and 3R/3R (homozygosity for 3 tandem repeat sequences in the 5′ UTR) genotypes with the MSI+ and MSI−, while these genotypes were associated with a higher TS expression. As a consequence we can hypothesise that patients bearing CRC with the MSI+, the 2R/2R genotype and with low TS levels could have a better prognosis and they could not be drug resistant.

Key Words: Colorectal cancer; Thymidylate synthase; Polymorphism; Microsatellite instability.

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INTRODUCTION

12–15 % of all sporadic CRC's show MSI phenotype and the MSI phenotype appears to be associated with better survival in patients receiving 5-FU based adjuvant chemotherapy.[1] The cellular target of 5-FU is TS; its levels vary considerably among tumors and the response to 5-FU is influenced by the intratumoral activity of the enzyme, with high levels generally being associated with a poor response.[2] TS expression has been shown to be influenced by a polymorphic tandem repeat sequence in the 5′UTR. Increasing the number of repeats leads to an increase in TS expression[3] and to a worse response to chemotherapy. A recently detected 6 bp deletion polymorphism in the 3′UTR of the TS gene might also influence transcriptional and/or translational efficiency or mRNA stability.[4] The aim of our study was to investigate whether there was a correlation between the MSI phenotype and the TS mRNA levels in CRC.

MATERIALS AND METHODS

Formalin-fixed, paraffin embedded samples retrieved from archival material, of 54 patients affected by primary colorectal cancer previously untreated, were reviewed. MSI status was assessed using microsatellite markers recommended as a reference panel by the National Cancer Institute workshop on microsatellite instability in cancer detection in 1997.[5] The 5′ UTR Polvmorphism was evaluated amplifying DNA by Polymerase chain reaction, and amplification products were electrophoresed in 3% agarose gel. Products of 116 bp (2R/2R), and 144 bp (3R/3R) or both of these products (2R/3R), depending on the TS genotype were obtained. The 3′ UTR analysis was carried out by RFLP as previously described by Ulrich et al.[4]

The quantification of TS expression, was obtained by Light Cycler- TS mRNA quantification Kit (Roche), and calculation of the relative amount of TS m-RNA compared to the reference gene was done using the Light Cycler Relative Quantification Software (Roche).

RESULTS

A significant association was found between the MSI status and the homozigosity for the double repeat patients, while no significant association was found between MSI status and the presence or absence of 6 bp- insert. The TS mRNA expression showed significantly different values in tumours with increasing copy number of repeat (2R/2R vs. 2R/3R vs. 3R/3R). Furthermore significantly higher values were found in the 3R/3R group and 2R/3R group compared with 2R/2R respectively. No significant association was found for the polymorphism of the 3′UTR and the TS mRNA levels.

DISCUSSION

Adjuvant chemotherapy with 5-FU improves survival in a subgroup of CRC patients but predictive markers are required to identify patients that benefit from such

treatment. MSI was found to be associated with a more favorable outcome, so as low intratumoral TS expression levels were associated with a good response to chemotherapy. It has been described in previous studies that TS expression depends on the genotype of the 5′UTR enhancer region of the corrisponding gene. The genotype 2R/2R is more frequently associated with MSI and lower TS levels. As consequence we can hypothesise that patients bearing colorectal cancer with MSI, the 2R/2R genotype and with low TS levels could have a better prognosis and could not be drug resistant.

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