

**FUNCTIONAL *MDR1* POLYMORPHISMS (G2677T AND C3435T) AND
TCF4 MUTATIONS IN COLORECTAL TUMORS WITH HIGH
MICROSATELLITE INSTABILITY**

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Abstract: The multidrug resistance 1 (*MDR1*) gene and transcription factor 4 (*TCF4*) gene are suggested to be involved in the WNT signalling pathway, the most important pathway altered in colorectal cancer. Mutations in both genes have been identified and associated with colorectal tumors exhibiting high microsatellite instability (MSI-H). In this study, we report on the distribution of functional polymorphisms in the *MDR1* gene and somatic frameshift mutations in the *TCF4* gene coding mononucleotide repetition in 62 MSI-H colorectal tumors. Somatic frameshift mutations in (of) the *TCF4* gene were identified in 24/62 (39%) of the studied MSI-H tumors. The estimated allele frequencies of functional polymorphisms in (of) exon 21 (2677 G>T, Ala893Ser) and exon 26 (3435 C>T, Ile1142Ile) of the *MDR1* gene were 0.42 and 0.46 in the controls and 0.54 (p=0.035) and 0.60 (p=0.017) in the MSI-H tumors. However, the allele frequency of both functional *MDR1* polymorphisms did not significantly differ between MSI-H tumors with *TCF4* mutations and those without. These results support the involvement of the *MDR1* gene in the tumorigenesis of MSI-H tumors and also suggest that functional polymorphisms in the *MDR1* gene and mutations in the *TCF4* gene are likely to occur independently in MSI-H tumors.

Keywords: Microsatellite Instability, *MDR1* Mutations, *MDR1* Polymorphisms, *TCF4* Mutations, Colorectal Cancer

INTRODUCTION

Microsatellite instability (MSI) is characteristic for a proportion of sporadic colorectal cancers (CRC), and for 90% of cancers from patients with Hereditary non-polyposis colorectal cancer (HNPCC) (reviewed in [1]). In MSI tumors, frequent somatic mutations have been identified in genes coding mononucleotide repeats, including *TCF4* transcription factor [2], involved in the WNT signalling pathway, the most important pathway altered in colorectal cancers. Recently, a transcription factor complex TCF4/ β catenin responsive element was identified in the promoter region of the *MDR1* gene, suggesting *MDR1* as the downstream target of the WNT signalling pathway [3]. *MDR1* codes for P-glycoprotein (P-gp), an ATP dependent transmembrane transporter that was initially identified by its overexpression in human tumor cells after

cancer chemotherapy where it was associated with multidrug resistance [4]. We have recently identified naturally occurring *MDR1* mutations associated with MSI-H colorectal tumors [5].

In order to ascertain the mutual role of *MDR1* and *TCF4* in the development of a subset of CRC, we screened MSI-H tumors for *TCF4* somatic frameshift mutations and *MDR1* functional polymorphisms.

MATERIALS AND METHODS

Analysis of microsatellite instability

Microsatellite instability (MSI) analysis using a “reference panel” of microsatellite markers was performed on 630 Slovenian unselected primary colorectal cancers (CRC) as described in our previous study [6]. Sixty-two tumors were determined as high microsatellite unstable tumors (MSI-H) and analysed for *TCF4* mutations and *MDR1* functional polymorphisms.

MDR1 and *TCF4* mutational analysis

We amplified exons and exon/intron boundaries 21 and 26 of the *MDR1* gene in a 10 μ L PCR reaction (10 minutes at 95°C followed by 35 cycles (of): 30 seconds at 95°, 30 seconds at 55°, 30 seconds at 72° and 7 minutes at 72°) containing 50 ng purified genomic DNA, 200 μ M each primer, 200 μ M each deoxynucleoside triphosphate, 1,5 mM MgCl₂, 1 X PCR buffer and AmpliGold polymerase (Perkin Elmer Cetus, Norwalk, CT). The primers used were 5'-TGCAGGCTATAGGTTCCAG (forward) and 5'-TTTAGTTTGACTCACCTTCCC (reverse) for exon 21, and 5'-ATCTCACAGTAACTTGGCAG (forward) and 5'-TCAAACCTATAGGCCAGAGAG (reverse) for exon 26. We amplified part of the exon 17 region including (A)₉ repetitions of the *TCF4* gene using previously reported primers [2]. For mutational analysis of the *MDR1* and *TCF4* genes, we used a non-isotopic conformation analysis consisting of Single Strand Conformational Polymorphism (SSCP), heteroduplex (HD) and Double Strand Conformation (DSC) analyses. After electrophoresis on polyacrylamide gels, the DNA was silver stained. Sequencing was performed with a BigDye Terminator Cycle Sequencing Ready Reaction Kit and an ABI 310 sequencer (Perkin Elmer Cetus, Norwalk, Conn., USA).

Statistical analysis

The results of the χ^2 test were calculated using SPSS software to compare the allele frequencies of *MDR1* polymorphisms between MSI-H tumors and the controls (healthy unrelated blood donors). In all the tests, P values of less than 0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

We screened MSI-H tumors for the common *MDR1* polymorphisms, 2677 G>T, (Ala893Ser) and 3435 C>T (Ile1142Ile), previously shown to be associated with altered P-glycoprotein expression and activity in colon tissue [7]. We found significantly higher frequencies of both studied polymorphisms in patients with MSI-H CRC tumors than in the normal controls (Table 1, p=0.035 and p=0.017, respectively). These results support the theory of the involvement of the *MDR1* gene in the tumorigenesis of MSI-H tumors, also suggested in our previous study, where we described 7 different unique germline and somatic *MDR1* mutations in 6/38 MSI-H tumors [5]. To ascertain the role of *MDR1* in the development of CRC, we further analysed MSI-H tumors for *TCF4* mutations since a direct link between the *MDR1* and *TCF4* genes was suggested in CRC tumorigenesis via the WNT signalling pathway [2]. We identified somatic frameshift mutations in the *TCF4* gene in 24/62 (39%) of the studied MSI-H tumors, but no significant correlation could be determined between *MDR1* functional alleles and *TCF4* mutations. Of the six MSI-H tumors with unique *MDR1* mutations identified in our previous study [5], three had also *TCF4* mutations. Although additional MSI-H tumors with *TCF4* mutations should be analysed for *MDR1* functional polymorphisms and mutations to exclude the correlation between *MDR1* and *TCF4* alterations, it is likely that *MDR1* functional alterations and *TCF4* mutations occur independently in MSI-H tumors.

Tab. 1. Aomparison of *MDR1* allele frequencies and *TCF4* mutations in MSI-H tumors and controls; N=number of chromosomes analysed, NS= not significant

MDR1 polymorphism	Controls N=200	MSI-H N=124	P controls vs MSI-H	MSI-H with TCF4 mutations N=48	MSI-H without TCF4 mutations N=76	P MSI-H with vs without TCF 4 mutations
Exon 21, 2677 G>T						
allele G	0.58	0.46	0.035	0.44	0.47	NS
allele T	0.42	0.54		0.56	0.53	
Exon 26, 3435 C>T						
allele C	0.54	0.40	0.017	0.38	0.41	NS
allele T	0.46	0.60		0.62	0.59	

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