Polymorphism in MHC Class II Transactivator Gene is Not Associated with Susceptibility to Colorectal Cancer in Swedish Patients

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Abstract. Background: Reduced expression of major histocompatibility complex class II (MHC-II) genes in colorectal cancer (CRC) has been reported. MHC-II transactivator (CIITA), encoded by the MHC2TA gene, is considered to be the master regulator for MHC-II gene expression. A functional single nucleotide polymorphism (SNP) –168A→G in the promoter region of the MHC2TA gene is suggested to have an influence on different autoimmune diseases. Patients and Methods: Our study was performed to evaluate the association between the –168A→G MHC2TA gene variant in patients with CRC versus a control group. Using the TaqMan system, this SNP was screened in 248 CRC patients and 256 controls. Results: No significant difference in genotype distribution or in allelic frequencies was found between the two groups, nor any association with clinical characteristics. Conclusion: The results of this study suggest that –168A→G polymorphism of the MHC2TA gene is not associated with susceptibility to CRC.

Lack of expression of MHC-II genes is associated with tumour growth and failure to mount an immune response (6-8). Patients with bare lymphocyte syndrome (BLS) immunodeficiency reveal the absence of MHC-II protein which can be caused by mutations in transcription factors that regulate MHC-II gene expression (4, 9, 10). One such factor is a non-DNA binding coactivator called class II transactivator (CIITA), encoded by the MHC2TA gene, which interacts and stabilizes other transcription factors on the MHC-II promoter region and is considered to be the master regulator for MHC-II gene expression (2, 11, 12).

Major histocompatibility class II (MHC-II) genes encode cell-surface glycoproteins that present processed antigen to CD4+ T lymphocytes (1). In humans, three glycoproteins are expressed, polymorphic human leukocyte antigen HLA-DR, HLA-DP and HLA-DQ (2-4). These MHC-II isotypes are normally expressed constitutively on restricted cell types, including macrophages, dendritic cells, B lymphocytes and thymic epithelium. A wide variety of other cell types can be induced to express MHC-II antigens by different cytokines (2-5).

Single nucleotide polymorphisms (SNPs) have been detected in the promoter region of the gene encoding human CIITA which is mapped to chromosome 16p13 (15, 16). One of them –168A→G, is associated with autoimmune Addison’s disease (17). Moreover, –168A→G SNP has been shown to be associated not only with other autoimmune diseases such as rheumatoid arthritis and multiple sclerosis but also with myocardial infarction (18). It has been suggested that the genotype GG leads to reduced expression of CIITA and MHC-II proteins accordingly (18).

Based on the suggested role of the functional gene polymorphism –168A→G in MHC2TA, an examination of this SNP was performed to evaluate its influence on CRC.

Patients and Methods

Patients and controls. This study comprised blood samples from 248 consecutive patients from southeastern Sweden who underwent surgical resections for primary colorectal adenocarcinomas at the Department of Surgery, Ryhov County Hospital, Jönköping, Sweden.
The patient group represented 127 males and 121 females with a mean age of 70 years (range 29-93 years). The tumours were localized in the colon (n=124) and rectum (n=124) and were classified according to Dukes’ classification system: stage A (n=47), stage B (n=102), stage C (n=85) and stage D (n=14). Blood donors (n=256) with no known CRC history and from the same geographical region as the CRC patients were selected as the controls. The control group consisted of 136 males and 120 females with a mean age of 68 years (range 50-83 years). All blood was centrifugated to separate plasma and blood cells and then stored frozen at −78°C.

DNA extraction and genotyping. DNA was isolated from blood using a QiaAmp DNA Blood Kit (Qiagen, CA, USA). DNA samples were genotyped using the 5'-exonuclease allelic discrimination assay (Applied Biosystems, CA, USA). Taqman SNP Genotyping Assay was used for analysis of the rs3087456 (CIITA, –168A→G) genotype (Applied Biosystems). Ten nanograms DNA was amplified in a total volume of 12 μl containing TaqMan Universal PCR Master Mix (Applied Biosystems) including 1x TaqMan SNP Genotyping Assay. Amplification was performed using an initial cycle at 50°C for 2 min followed by 1 cycle at 95°C for 10 min and finally 40 cycles at 95°C for 15 s and 60°C for 1 min. A post PCR endpoint reading was performed on each plate using the 7500 Fast Real-Time PCR System (Applied Biosystems). The manual calling option in the allelic discrimination application ABI PRISM 7500 SDS software version 1.3.1 (Applied Biosystems) was then used to assign genotypes.

Statistical analysis. Differences in the frequencies of the MHC2TA polymorphism between CRC patients and the control group and between clinical characteristics within the CRC subgroup were analyzed using the Chi-square test and the Hardy-Weinberg equilibrium was tested for the genotypes. Statistical analysis was performed using SPSS for Windows computer package (Rel. 14.0, 2005; SPSS Inc., Chicago, IL, USA). Results were considered significant at p<0.05.

Results

To analyse the influence of the MHC2TA gene variant on colorectal carcinogenesis, the prevalence of promoter –168A→G gene polymorphism in 248 CRC patients and 256 controls was investigated by a TaqMan allelic discrimination system. There was no significant difference in genotype distribution or in allelic frequencies between CRC patients and controls (Table I). Subdividing the patients into group of colonic and rectal cancer, or localized Dukes A+B and disseminated Dukes C+D disease, we were also unable to detect any significant difference (Table II). Genotype and allelic distributions in CRC patients and the control group were not associated with other clinical characteristics such as age and gender (data not shown). Neither the patient nor the control group showed significant deviation from the Hardy-Weinberg equilibrium.

Discussion

MHC-II protein plays a vital role during inflammation by presenting antigens to CD4+ T-cells and in presenting tumour-derived antigens, and hence in activating and regulating the antitumour response (1). Local immunoregulation mediated by infiltration of inflammatory cells into CRC tissue is considered of importance for tumour progression (19) and there is evidence that cytokines may contribute to malignant progression (20). In addition, it has been reported that MHC-II-negative CRC tissue exhibit a lower grade of T-cell infiltrate and thereby escape from immune surveillance (14).

Altered expression of MHC-II in human CRC has been described. Existing data on MHC-II expression in human CRC show that normal colorectal tissue displays weak to high grade of MHC-II proteins and that CRC shows reduced expression compared to adjacent normal tissue (13). Whether suppressed MHC-II expression contributes to colorectal carcinogenesis remains unknown.

The coactivator CIITA is referred to as the master regulator of MHC-II gene transcription and is important for both constitutive and cytokine induced expression in a variety of cell types (12). There is evidence that reduced expression of MHC-II gene results from epigenetic silencing by hypermethylation of MHC2TA in several types of cancer (21). A single nucleotide polymorphism (SNP), –168A→G, has been detected in the promoter region of the gene.

### Table I. Genotypic and allelic distributions in % (n) of MHC2TA polymorphism in CRC patients and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CRC (n=248)</th>
<th>Controls (n=256)</th>
<th>Allele</th>
<th>CRC (n=496 alleles)</th>
<th>Controls (n=512 alleles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>–168A→G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>56.8 (141)</td>
<td>58.6 (150)</td>
<td>A</td>
<td>75.2 (373)</td>
<td>77.0 (394)</td>
</tr>
<tr>
<td>A/G</td>
<td>36.7 (91)</td>
<td>36.7 (94)</td>
<td>G</td>
<td>24.8 (123)</td>
<td>23.0 (118)</td>
</tr>
<tr>
<td>G/G</td>
<td>6.5 (16)</td>
<td>4.7 (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CRC patients vs. controls: genotypes overall p=0.680, alleles p=0.515.

### Table II. Genotype and allele numbers of the MHC2TA gene polymorphism (–168A→G) regarding to location and Dukes’ stage in CRC patients.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>76</td>
</tr>
<tr>
<td>A/G</td>
<td>43</td>
</tr>
<tr>
<td>G/G</td>
<td>5</td>
</tr>
</tbody>
</table>

Colon (n=124) 76 43 5 195 53

Rectum (n=124) 65 48 11 178 70

Dukes’ A+B (n=149) 87 53 9 227 71

Dukes’ C+D (n=99) 54 38 7 146 52

Colon vs. rectum: genotypes overall p=0.184, alleles p=0.077. Dukes’ A+B vs. Dukes’ C+D: genotypes overall p=0.827, alleles p=0.538.
encoding human CIITA (15, 16). In a recent study, Swanberg et al. (18) showed that this SNP was associated with rheumatoid arthritis and multiple sclerosis in Swedish patients. However, another result in German patients does not reveal this association (22).

Functionally, −168A→G MHC2TA polymorphism may have an effect on CRC progression as a regulator of the MHC-II expression. The results of our study, however, demonstrated that there was no significant difference in genotype distribution or in allelic frequencies between CRC patients and control subjects and no association with clinical characteristics. Thus the −168A→G polymorphism of the MHC2TA gene seems not to be a useful tumour marker that reflects clinical outcome of CRC. In a forthcoming study, we intend to investigate the influence of this SNP on the 5-year survival rate of the CRC patients.

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References


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