RESEARCH ARTICLE

The SOCS-1 -1478CA/del Polymorphism is not Associated with Colorectal Cancer or Age at Onset in Turkish Subjects

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Abstract

Background: Suppressor of cytokine signaling (SOCS)-1 acts as a key regulator of many cytokine signaling pathways and its abnormal expression has been identified in several human malignancies, suggesting potential roles in carcinogenesis. The aim of this study was to investigate any association between the functional SOCS-1 -1478CA>del polymorphism and colorectal cancer (CC) as well as age at onset in a Turkish clinical sample. Materials and Methods: A total of 122 subjects were enrolled in this case-control study (70 CC cases and 52 controls). The SOCS-1 -1478CA>del polymorphism was genotyped using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Results: The odds ratio of the del allele for CC relative to the CA allele was not significantly different between the groups (OR=0.71, 95% CI=0.41–1.22, p=0.27). This result did not change after adjustment for age and sex on multivariable regression analysis (OR=0.84, 95% CI=0.59–1.34, p=0.53). When the SOCS-1 -1478CA>del polymorphism was analyzed among CC patients in relation to the age at disease onset, we found no significant differences between subjects with the del/del, CA/del, and CA/CA genotypes. Conclusions: The results of our study did not point towards a major role of the SOCS-1 -1478CA>del polymorphism in the pathogenesis of CC in Turkish subjects.

Keywords: Colorectal cancer - age at onset - suppressor of cytokine signaling - polymorphism - association study

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Introduction

Colorectal cancer (CC) is the third most common cancer and one of the leading causes of cancer deaths in developed and developing countries (Haggar and Boushey, 2009; Cunningham et al., 2010). A complex interplay of multiple environmental and genetics factors is involved in the pathogenesis of CC, although the interactions of such events may vary from one individual to another (Goel and Boland, 2010). From a pathological standpoint, rhe development of CC is characterized by a sequence of events during which normal colonic epithelium gradually transforms to carcinoma tissue, in most cases via the development of colorectal adenomas (Brenner et al., 2007; Carvalho et al., 2012). This sequence of events seems to be driven by an accumulation of molecular alterations causing progressive disorders in cell growth, differentiation, and apoptosis (Sugai et al., 2003; Carvalho et al., 2012). Genetic factors are believe to play a major role in colorectal carcinogenesis (Fearon, 2011; Armaghany et al. 2012). Although dominantly inherited high penetrance genes are known to cause hereditary CC, the majority of colorectal cancers are sporadic; consequently, individual differences in susceptibility are likely to depend on a series of low penetrance genes (Aiello et al., 2011; Castellví-Bel et al., 2012).

Evidence derived from experimental and epidemiological studies has suggested that chronic inflammation may play a key role in the transition from normal colonic epithelium to the development of malignancy (Terzić et al., 2010; Ullman and Itzkowitz, 2011). Of the many risk factors associated with CC, several-including ulcerative colitis (Eaden et al., 2001), colonic Crohn's disease (Freeman, 2008), and low-grade inflammation in response to the microbial gut flora (Arthur and Jobin, 2011)-may contribute to a state of prolonged exposure to inflammation. Given that inflammatory process are influenced by inflammation-related genes, functional genetic polymorphisms in inflammatory pathways may also influence the risk of CC (Boland, 2010; Coghill et al., 2011).

Suppressor of cytokine signaling (SOCS)-1, also called JAK binding protein-1 or STAT-induced STAT inhibitor-1, is an essential regulator of many cytokine signaling pathways (Ohya et al., 1997), including those upregulated in the inflamed colonic mucosa. In particular, evidence suggests that SOCS-1 acts as a critical regulator of the IFN-γ (Piganis et al., 2011) and IL-4 (Losman et al., 1999) signaling pathways, which are both involved in the pathogenesis of CC (Landi et al., 2007; Slattery et

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al., 2011). A functional SOCS-1 promoter polymorphism (-1478CA>del) has been shown to increase SOCS-1 transcription *in vitro* (Harada et al., 2007). Notably, a high tumor-specific expression of SOCS1 has recently suggested a direct tumor-promoting function of SOCS1 (Zhang et al., 2012). In light of this evidence, we sought to investigate whether the SOCS-1 -1478CA>del polymorphism is associated with CC susceptibility and its age at onset in a Turkish clinical sample.

Materials and Methods

Study participants

The study sample for this case-control study consisted of 122 unrelated subjects of Turkish descent, including patients with CC (CC cases, n=70) and subjects without CC (controls, n=52). All of the patients in the CC group underwent surgery and had a pathologically-confirmed diagnosis. The control subjects were apparently healthy subjects, without a personal or family history of CC and no lower abdominal symptoms, diarrhea, or hematochezia. The local Ethics Committees approved the study, and written informed consent was obtained from all participants. The study was conducted in compliance with the tenets of the Declaration of Helsinki.

Genotyping

Genomic DNA was purified from peripheral blood samples using the QiaAmp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. The procedure for detecting the SOCS-1 -1478CA>del polymorphism was based on PCR amplification (forward primer: 5'-TGTCGTCCAGCTGCACCTC-3'; reverse primer: 5'-ACCACAGGCTTCAGAGGAAC-3'), restriction cleavage with DdeI (New England Biolabs, Ipswich, MA, USA) and separation of the DNA fragments by 3% agarose gel electrophoresis [figure1], as previously described (Harada et al., 2007). For quality control, genotyping analyses were done blind with respect to case/control status, and a random 25% of the samples were repeated. The concordance rate for duplicate genotyping was 100%. Two investigators independently reviewed all results.

Data analysis

All statistical analyses were performed using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) for Windows, except for statistical power, which was calculated using with the StatMate software, version 2.0 (GraphPad, San Diego, CA, USA). Continuous data were expressed as mean±SD and compared using the Student's t-test or analysis of variance (ANOVA), as appropriate. Genotypes were obtained by direct counting followed by allele frequency calculations. We tested the Hardy-Weinberg equilibrium using online resources (http://www.husdyr.kvl.dk/htm/kc/popgen/genetik/applets/kitest.htm) (Emanuele et al., 2010). Genotype frequencies and categorical variables were compared using the χ^2 test with α set at 0.05 (two-tailed). We used multivariable logistic regression analysis to analyze the association between the SOCS-1 -1478CA>del and CC after adjusting for age and sex. Results were expressed as odds ratios (OR) with their 95% confidence intervals (CI).

Results

The mean age was significantly lower in controls (30.4±5.7 years) than in CC patients (59.9±11.2 years, P<0.001). Moreover, the prevalence of male sex was found to be significantly different between controls (21/31) and CC cases (46/24, p<0.01). Table 1 displays the distribution of the SOCS-1 -1478CA>del polymorphism in CC cases and controls. Genotype frequencies in our sample were in Hardy-Weinberg equilibrium both in CC patients (p=0.30) and controls (p=0.38). The odds ratio of the del allele for CC relative to the CA allele was not significant (OR=0.71, 95% CI=0.41–1.22, P=0.27). These results did not change after adjustment for age and sex in multivariable regression analysis (OR=0.84, 95% CI=0.59–1.34, p=0.53).

When the SOCS-1 -1478CA>del polymorphism was analyzed among CC patients in relation to the age at disease onset, we found no significant differences (ANOVA test, p=0.26), between subjects with the del/del (55.3±17.1 years), CA/del (55.5±10.7 years), and CA/CA (59.9±10.4 years) genotypes.

Table 1. Distribution of the SOCS-1 -1478CA>del Polymorphism in Patients with Colorectal Cancer and Healthy Controls

	CC cases (n=70)	Controls (n=52)
del/del	6 (8.6%)	10 (19.2%)
CA/del	30 (42.9%)	19 (36.5%)
CA/CA	34 (48.5%)	23 (44.3%)
OR (95% CI) [del vs. CA]	0.71 [0.41-1.22], p= 0.27	

OR = odds ratio; CI = confidence interval.

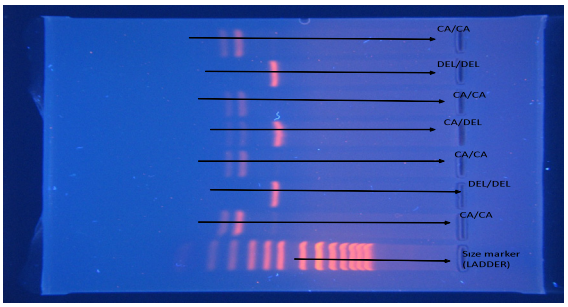

Figure 1. Agarose gel electrophoresis showing genotypes of the SOCS-1 -1478CA>del Polymorphism. Bands in 3% gels stained with ethidium bromide fluorescence under ultraviolet light

Discussion

The results from this study indicate that the SOCS-1 -1478CA>del polymorphism was associated with neither susceptibility to CC nor with its age at onset in Turkish subjects. For the purpose of this study, we selected this well-defined single nucleotide polymorphism as it has been previously shown to have functional consequences (Harada et al., 2007). Based on the observed prevalence of the minor del allele, our study had 80% power to detect

a relative risk of 1.67 with a significance level (alpha) of 0.05 (two-tailed) between CC cases and controls.

In the present study, we found significant differences in the SOCS-1 -1478CA>del polymorphism frequency between our control subjects and those of Chan et al. (2010), which represented genotype distributions in the Taiwanese population. Moreover, our control genotype frequencies were also different from those obtainedin Japanese by Harada et al. (2007). Our higher frequency of the del allele in control subjects suggests the presence of ethnic differences in SOCS-1 genotype distribution. In our study, the distribution of the SOCS-1 -1478CA>del polymorphism was in Hardy-Weinberg equilibrium, suggesting the absence of genotyping errors and/or selection bias. It has been previously suggested that SOCS-1 is involved in the inflammatory processes occurring in the colonic mucosa (Chinen et al., 2006), and that the -1478CA>del variant is a functional polymorphism (Harada et al., 2007). As is the case for any malignancies, other factors may be involved in the pathogenesis of CC besides genetic predisposition, including environmental, and host factors (Fernandez et al., 2004; Lin, 2009). To the best of our knowledge, this study is the first to investigate the SOCS-1 -1478CA>del variant in relation to the risk of CC. However, SOCS-1 is a reasonable candidate gene for CC because aberrant methylation of this gene has been observed in younger colorectal cancer patients (Fujitake et al., 2004). Similarly, an abnormal aberrant promoter methylation of SOCS-1 has been observed in CC tissues than in non-cancerous tissues (Lin et al., 2004). Interestingly, spontaneous colorectal cancers have been shown to develop in Socs-1 deficient mice (Hanada et al., 2006).

In the interpretation of our findings, several caveats merit consideration. First, the number of subjects was relatively small. We thus believe that our findings should be considered explorative, and need to be confirmed in larger sample sizes. Second, as our study cohort consisted only of Turkish subjects without ethnical diversity, these results require replication in different ancestry populations. Third, it should be noted that our study shares the limitations of case-control investigations with regard of population stratification risk. Moreover, because we did not have information on environmental factors such as eating habits and alcohol consumption, we could not assess the interactions between those environmental factors and the SOCS-1 -1478CA>del polymorphism. Finally, we are aware that the use of only one single nucleotide polymorphism in this report negates the exploration of significant interactions among distinct polymorphisms in a given haplotype. As several polymorphisms have been identified in the SOCS-1 gene (Gylvin et al., 2009), a larger number of genetic markers should be examined to shed more light on the putative association of this gene with CC.

In summary, the results of our study did not point toward a major role of the SOCS-1 -1478CA>del polymorphism in the pathogenesis of CC. Indeed, no association of this common genetic variant with the diagnosis of CC was discerned. Moreover, this polymorphism was not found to be associated with the age at disease onset among our CC patients. Interestingly, our results indicate the presence of a ethnic heterogeneity in the distribution of the SOCS-1 -1478CA>del polymorphism between Turkish and Asian populations.

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