SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 3: 517-519, 2012

Efficient association analysis between colorectal cancer and allelic polymorphisms of *HLA-DQB1* by comparison of age of onset

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Received September 16, 2011; Accepted December 19, 2011

DOI: 10.3892/ol.2011.540

Abstract. Common methods for identifying cancer-related genes are solely based on differences between gene frequencies in the disease and control groups, and do not take into account the age of onset in the gene carriers. In the present investigation, we developed a new study design based on the age of onset of cancer for the identification of colorectal cancer-related genes. The samples from patients with colorectal cancer were typed using an HLA-DQB1 polymerase chain reaction using a sequence-specific primers (PCR-SSP) typing kit. The mean age of subjects with and without the alleles was calculated. The mean age of subjects with the HLA-DQB1*02 allele was significantly less than that of subjects without this allele ($p<0.05$). We found that the HLA-DQB1*02 allele was associated with colorectal cancer susceptibility. This new method of analysis may therefore be an efficient and reliable approach for the identification of cancer-causing genes.

Introduction

Genetic linkage analysis has been highly successful in mapping the genes responsible for Mendelian diseases. During the past decade, attempts have been made to extend this approach to multifactorial disorders and other health-related traits. However, since complex diseases, including colorectal cancer, are characterized by the modest contributions of each susceptibility gene, identification of strong and replicable linkages has proven to be difficult (1,2). In this regard, the results of our linkage studies on human leukocyte antigen (HLA) allele polymorphisms and cancers have also been discouraging owing to inconsistent findings and weak linkage signals; however, these studies have indicated that genetic factors may affect the early onset of cancer. Therefore, we developed a new study design based on the age of onset of cancer.

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Key words: colorectal cancer, gene identification, human leukocyte antigen, complex disease

Cancer is typically a complex disorder to which, until now, an unknown number of genes contribute by interacting with each other and the environment (3,4). The identities of these genes have remained elusive in spite of the rapid pace of development of molecular technology and the increase in genome sequence information. This scenario may be partly attributed to by the absence of methods for high-efficacy analysis of genetic information. Single nucleotide polymorphisms (SNPs) are currently popular allelic markers (5,6); however, their value is largely qualitative. They may not be satisfactory indicators of complex diseases owing to their low quantitative efficacy. Our new study design uses the age of onset of cancer as an indicative factor that may provide a quantitative rather than a qualitative estimate (by assessing allele frequency at the locus). The efficacy of analysis may be improved since a quantitative measure is more statistically robust than a qualitative one.

The HLA complex genes are located on the short arm of chromosome 6 and are the most polymorphic loci within the human genome. The primary function of HLA is to stimulate the immune system to identify infectious pathogens and eliminate them. The status of HLA alleles is important in immune responses and immunological tolerance (7). Specific antigens of the HLA system are associated with numerous solid tumors (8-10). Specifically, persistent pro-tumor immune responses, now generally accepted to potentiate primary tumor development, are being recognized as mediators of cancer metastasis (11-13). The aim of the present study was to determine whether polymorphisms in the HLA-DQB1 alleles are associated with colorectal cancer and to identify an efficient research method for the identification of cancer-related genes.

Materials and methods

Subjects. A total of 100 Chinese patients (200 chromosomes), 52 males and 48 females with a mean age of 61.77±11.42 years, who wished to participate in the study, were included. Patients with colorectal cancer were evidenced by surgical intervention and their ages at the time of first surgery were recorded. All patients gave written informed consent, and the study was approved by the Institutional Ethics Committee of Dalian Medical University.

HLA-DQB1 allele typing. Genomic DNA was extracted from white blood cells using standard techniques for HLA typing.

Table I. Average age differences between subjects with and without certain HLA-DQB1 alleles.

DQB1*	Subjects with the allele		Subjects without the allele		p-value
	n	Average age	n	Average age	
02	43	57.49±12.71	157	62.94±10.79	**0.005**
03	73	63.03±10.10	127	61.05±12.09	0.239
04	12	61.42±8.50	188	61.79±11.60	0.912
05	16	64.63±14.63	184	61.52±11.11	0.298
06	56	62.68±11.06	144	61.42±11.57	0.484

Bold text indicates a significant difference after Bonferroni correction; p<0.01 (0.05/5).

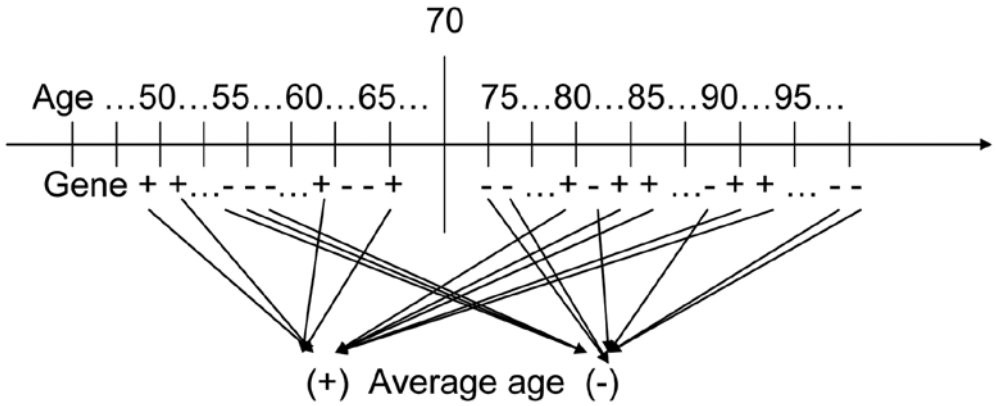

Figure 1. Diagram of the experimental design.

The samples were typed using an *HLA-DQB1* 'low resolution' polymerase chain reaction using a sequence-specific primers (PCR-SSP) typing kit (Pel-Freez Clinical Systems, Brown Deer, WI, USA) including allele-specific primers for DQB1*02, DQB1*03, DQB1*04, DQB1*05 and DQB1*06. The commercial tests were run according to the manufacturer's instructions. Products were separated by electrophoresis in 2% agarose gel and visualized by ethidium bromide staining and ultraviolet (UV) transillumination. Automated gel reading was performed using Pel-Freez software.

Experimental design and statistical analysis. The basic concept of the new analysis method is to consider the age of onset as a pathogenic effect of a gene. By comparing the average ages of subjects carrying a gene and those not carrying the gene, the association between the gene and the disease may be ascertained. Fig. 1 shows the experimental design.

In the present study, the age of the subject was considered to be the dependent variable, and the gene carriers (subjects carrying or not carrying the gene) were considered to be the grouping variable. A Student's t-test was used to assess the difference between the mean ages of the subjects that carry the genes and those that do not carry the genes. P<0.05 was considered to indicate a statistically significant difference (two-tailed test), and a Bonferroni correction was applied. If the average age of the subjects who carried a certain gene was significantly lower or higher than that of the subjects who did not carry the gene, then the gene was regarded as a disease-related gene (susceptibility or resistance gene). Analyses were performed using the SPSS 13.0 statistical software package.

Results

A total of 100 Chinese patients were included in this study, and the age at onset of colorectal cancer was observed. The patient samples were typed using a HLA-DQB1 PCR-SSP typing kit. The average ages of the patients with and without the alleles were calculated. Results showed that the mean age of subjects with the HLA-DQB1*02 allele was less than that of subjects without this allele (p<0.05), as shown in Table I. No significant differences were observed between the mean ages of the subjects with and without other HLA-DQB1 alleles.

Discussion

Studies regarding to the pathogenic effects of a gene on organisms should include factors such as the differences between the frequency distributions of the gene in the disease and the control groups (the stronger the effect of a gene, the greater the differences in frequency distribution) and early or late age of disease onset in gene carriers (the stronger the effects, the earlier the onset). However, the traditional methods for identifying disease-related genes have solely been based on differences between the gene frequencies in the disease

group and the control group. Owing to enumeration data, the frequency analysis requires large sample sizes and has low efficacy. Our new analysis method is a quantitative one, and its statistical efficiency is much higher than that of the traditional frequency analysis. Therefore, unlike the methods used in other studies, this method may be used to bypass the requirement for large sample sizes, thus improving analytic efficacy.

In this investigation, we adopted a reliable assay for *HLA-DQB1* genotypes; the procedures were performed in conditions that were in accordance with international standards. Our results indicated that the mean age of subjects with the HLA-DQB1*03 allele was less than that of subjects without the allele ($p<0.05$), which implied that this allele was associated with colorectal cancer susceptibility. These results suggest that persistent pro-tumor immune responses may change with the HLA allelic polymorphism and that the new analysis method is an efficient and reliable approach for the identification of complex disease-causing genes.

We expect that the increasing integration of genetics, epidemiology and clinical trials through the sharing of valuable data among laboratories may lead to new study methods that may identify colorectal cancer-related genes and also clarify their interactions with other risk factors.

Acknowledgements

The study was supported by grant LR201014 from the Program for Liaoning Excellent Talents in the University of China.

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