Journal of Dental Sciences (2014) 9, 41—48

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

HLA typing in Taiwanese patients with oral squamous cell carcinoma

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Received 23 February 2011; Final revision received 28 May 2011
Available online 22 March 2013

KEYWORDS
head and neck cancer;
HLA alleles;
polymorphism;
sequencing-based typing

Abstract *Background/purpose:* The human leukocyte antigen (HLA) system, which plays a vital role in immunity, is the most polymorphic gene complex found in the human genome. This study investigated HLA-related alleles and haplotypes in Taiwanese patients with oral squamous cell carcinoma (OSCC).
Materials and methods: HLA class I (HLA-A and HLA-B) antigens and class II (HLA-DRB1) alleles were determined in 105 patients with OSCC and compared with those in 190 healthy controls. The antigens were measured serologically and the alleles by sequencing-based typing.
Results: Compared with the control group, patients with OSCC had higher frequencies of HLA-A24, HLA-B54, HLA-DRB1*0405, and HLA-DRB1*1201, while they had lower frequencies of HLA-B58 and HLA-DRB1*1302. Haplotype frequencies also varied significantly in individuals with OSCC, with certain haplotypes associated with lymph node metastases or a particular tumor stage.
Conclusion: These results suggest that HLA genetic factors influence susceptibility to OSCC and perhaps to lymph node metastasis and tumor progression.

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http://dx.doi.org/10.1016/j.jds.2013.02.005

Introduction

The human leukocyte antigen (HLA) system, located on chromosome 6 and intimately involved in regulation of the immune response, is the most polymorphic gene complex found in the human genome. HLA class IA, B, and C antigens are expressed by almost all nucleated cells and platelets, and are recognized by CD^{8+} cytotoxic T cells. HLA class II DR, DQ, and DP molecules are expressed by antigen-presenting cells. Their function is to present exogenous antigens to CD^{4+} helper T cells. Obviously, quantitative changes in HLA expression may alter immune status. However, the extremely polymorphic nature of this portion of the genome also results in considerable interindividual differences. Several HLA types are associated with an increased risk of various immunologically medicated diseases.[1] There is also evidence that the HLA gene complex may mediate susceptibility to or protection from malignancies.[2,3]

Oral cancer ranks sixth in cancer incidence worldwide, but there are epidemiologic variations between different geographic regions. It is a leading form of cancer in most Asian countries and the fourth most common malignancy in men in Taiwan.[4] The frequent use of tobacco, alcohol, and betel quid among Asians likely accounts in part for regional variations in disease incidence. However, familial clustering of disease, linkage studies, and molecular findings also suggest that there may be specific genetic susceptibility among certain individuals. Alterations in immune function have been detected in patients with oral squamous cell carcinoma (OSCC),[5] along with frequent polymorphous autoimmune reactions to various tissue antigens.[6] The HLA complex has been implicated in the development of squamous cell carcinoma, particularly in head and neck tumors. Loss of heterozygosity in the HLA complex may provide tumor cells with an immune-escape phenotype. HLA class I expression in OSCC may regulate natural killer cell activity.[7–9]

We previously demonstrated an association between major histocompatibility complex class I chain-related gene A (MICA) polymorphism and OSCC. Genotyping of the HLA-A,B locus as well as MICA gene fragments in patients with OSCC may further improve our understanding of changes in immune function vis-à-vis the risk of developing this cancer. To the best of our knowledge, little is known about HLA typing in OSCC in Taiwan or Southeast Asia. Therefore, this study was designed to investigate the genotype and haplotype frequencies of HLA-A, HLA-B, and DRB1 in patients with OSCC.

Materials and methods

Study participants

Between November 2000 and December 2002, we recruited 105 consecutive patients with OSCC from the Oral and Maxillofacial Department at the Taipei Mackay Memorial Hospital. The diagnosis was made by histopathologically examining the biopsy specimens. A total of 105 patients described themselves ethnically as Min Nan (by a self-report). The control group included 190 participants identified as Min Nan, who visited our clinic for routine physical examination, minor operations for non-neoplastic disease, or who had maxillofacial trauma. In both study and control groups, individuals with autoimmune disorders, blood diseases, or a history of a previous malignancy were excluded. The participants were all unrelated to each other.

DNA extraction

Peripheral blood samples were drawn from all the participants of the study and the control groups. Genomic DNA was extracted from fresh or frozen peripheral blood leukocytes using the Pharmacia DNA isolation kit (Pharmacia Biotech, Germany).

HLADRB1 allele typing by sequence-based typing

The group-specific primers used for amplification of exon 2 of DRB1 alleles were modified from those used at the Diagnostic DNA Laboratory (Utrecht) and Tissue Typing Laboratory (Maastricht).[7] The Utrecht 5′ primers were used for DR1, DR2, DR8/12, and DR7 and the Maastricht primers for DR3/11/6, DR4, DR9, and DR10. All 5′ primers were located near the 5′ end of exon 2 containing an M13 (−21) sequence at the 5′ end for sequencing with the M13 sequencing primer. Because of a polymorphic site at position 270, a newly designed 3′ primer located at the intron 2—exon 2 junction (GCGCTCACCTCGCCGCTG) was used. When combined with the group-specific 5′ primers, this 3′ primer should amplify DRB1 alleles but not DR9. For DR9 amplification, the 3′ primer located at position 263—283 (CTCGCCGCTGCACTGTGAAG) was used. Two supplemental 3′-end primers, a TG primer (GCTGCACTGTGAAGCTCTCCA), and a GT primer (GCTGCACTGTGAAGCTCTCAC) located at position 257—276 were used to separate alleles when initial group-specific amplification failed to show the two alleles.

The amplification reaction mixture contained 50 ng genomic DNA, 15 mM Tris—HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM of each deoxyribonucleotide, 5% glycerol, 0.1 mg/mL cresol red, and 0.25 U of AmpliTaq Gold polymerase (Perkin Elmer, Foster City, CA, USA).[10,11] The polymerase chain reaction (PCR) was carried out using the GeneAmp PCR system (Perkin-Elmer Corporation, Foster City, CA, USA). The reaction mixture was subjected to denaturation at 95°C for 10 minutes followed by 32 cycles at 95°C for 10 seconds, 62°C for 30 seconds, 72°C for 30 seconds, and by a final extension at 72°C for 5 minutes. The PCR products were checked by 2% agarose gel electrophoresis, using 5 μL of reaction volume, and 1/10 of the diluted PCR products underwent direct sequencing using a BigDye Primer Cycle Sequencing Ready Reaction Kit sequence primer (−21M13) (Applied Biosystems, Foster City, CA, USA). Samples were then subjected to electrophoresis in an ABI 377 DNA sequencer and the results were analyzed by Match Tools and Sequence Navigator (Applied Biosystems, Foster City, CA, USA).

HLA-A, B serologic typing

Blood samples from all the patients and the control group were collected in acid citrate dextrose tubes and