Oral Oncology (2007) **143**, 232–240

available at www.sciencedirect.com

ScienceDirect

journal homepage: http://intl.elsevierhealth.com/journals/oron/

ORAL ONCOLOGY

HLA and MICA associations with head and neck squamous cell carcinoma

Judith Reinders [a], Erik H. Rozemuller [a], Henny G. Otten [b], Lars T.J.N. van der Veken [a], Pieter J. Slootweg [c], Marcel G.J. Tilanus [a,*]

[a] *Department of Pathology, University Medical Centre Utrecht, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands*
[b] *Department of Immunology, University Medical Centre Utrecht, P.O. Box 85500, 3508 GA, Utrecht, The Netherlands*
[c] *Department of Pathology, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB, Nijmegen, The Netherlands*

Received 25 January 2006; accepted 9 March 2006
Available online 20 July 2006

KEYWORDS
Head and neck squamous cell carcinoma;
HLA;
MICA;
Sequencing based typing

Summary Head and neck squamous cell carcinoma (HNSCC) is a very aggressive tumour arising from the epithelial lining of the upper aerodigestive tract. The precise mechanisms involved in the pathogenesis of HNSCC have not been elucidated. Previous studies observed aberrant HLA expression patterns on HNSCC tumour cells and this study focused on the allelic polymorphism of HLA genes and the MHC class I chain related gene A (MICA) and HNSCC. We investigated whether associations with HLA and/or MIC alleles or haplotypes are involved in the pathogenesis of HNSCC and could explain the observed HLA expression patterns. Patients and controls were typed for HLA-A, HLA-B, HLA-C, HLA–DRB1 and HLA–DQB1 with sequence specific priming (SSP), supplemented with sequencing based typing (SBT). MICA allelic polymorphism was included and MICA allele assignment was based upon the combination of high resolution SBT of exons 2–4 in combination with repeat analysis and nucleotide polymorphism of exon 5. HLA-B*35 ($p = 0.014$, OR = 0.31) and HLA-B*40 ($p = 0.013$, OR = 2.9) were significantly associated in respectively the metastasized patients and the oral cavity patients. In addition, the HLA-B*40–DRB1*13 haplotype ($p = 0.016$, OR = 4.1) was more often observed in the oral cavity patient group. The biological significance of the prevalence of specific HLA haplotypes in patients with oral cavity HNSCC and metastasizing HNSCC requires further investigation.

* Corresponding author. Address: Department of Pathology, H04.312, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. Tel.: +31 (0) 30 2506487/2507665; fax: +31 (0) 30 2544990.
E-mail address: M.Tilanus@umcutrecht.nl (M.G.J. Tilanus).

1368-8375/$ - see front matter
doi:10.1016/j.oraloncology.2006.03.003

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a solid epithelial tumour that occurs in general more often in males than in females. The majority of patients present with advanced stage of disease at time of diagnosis, deteriorating the prognosis for effective treatment, quality of life and survival.[1] Knowledge of the biological immune status of tumours is therefore important. This requires improvement and/or development of additional (immuno)therapeutical approaches to improve the prognosis for these patients.

The human leukocyte antigen (HLA) system is the human equivalent of the major histocompatibility complex (MHC) on the short arm of chromosome 6. Within the HLA extended region (together 7.6 Mb), approximately 250 genes are located including expressed genes and pseudogenes.[2] This region is comprised of three HLA regions: class I, class II and class III. Class I encode the classical HLA genes HLA-A, -B and -C and in the class II region HLA-DR, -DQ and -DP are located. In addition to class I and class II HLA genes, other HLA, HLA-related and non-HLA genes are located in these three classes, many of them involved in the immune system.[3]

The polymorphic MHC class I chain related gene A (MICA), an HLA-related gene and located centromeric of HLA-B, contains 6 exons, including a tri-nucleotide repeat polymorphism in the transmembrane region.[4–6] Associations with either of the repeats have been observed for several diseases, e.g. the MICA-A9 repeat is significantly associated with HNSCC, particularly in patients diagnosed with squamous cell carcinoma of the oral cavity, as previously observed.[7] MICA can either be constitutively expressed at the cell surface membrane or induced under abnormal conditions, e.g. tumour transformation. Several types of carcinomas express MICA on their cell surface. In some tumour types soluble MICA has been detected in the sera of cancer patients.[7–12] Dependent on whether MICA is expressed at the cell surface or as a soluble molecule, upon interaction with the NKG2D receptor, present on NK cells, $\gamma\delta$ T cells and $\alpha\beta$ CD8+ T cells, different immunological responses might occur. Tumour cell surface expression can activate the immune system while soluble MICA might oppose the immune system through blocking of the NKG2D receptor.[12–14]

The role of HLA in cancer is less obvious compared to its known roles in e.g. transplantation. In patients with nasopharyngeal carcinoma from Morocco and a few Asian countries, associations with several HLA alleles and/or haplotypes have been reported.[15–18] Increased and decreased risks for development of this type of tumour were observed, but it was not excluded that genes in linkage disequilibrium with the associated haplotypes may also be involved. We performed an exploratory study to investigate whether HLA alleles and/or HLA haplotypes are associated with HNSCC in Dutch patients. In addition we evaluated MICA allelic polymorphism, since the MICA gene is in linkage disequilibrium with HLA-B. Nasopharyngeal carcinoma is not a common type of tumour in the Netherlands, therefore this study focused on other tumour locations.

Material and methods

Patients and controls

One hundred and forty Dutch HNSCC patients, 98 male and 42 female with known specified tumour location, age of patients at the time of surgery, absence or presence of metastases and histological grade, and 106 Dutch healthy controls were included in this study. The 140 HNSCC patients can be grouped according to the location of the tumour: oral cavity group ($N = 57$), oropharynx group ($N = 28$), larynx group ($N = 39$) and hypopharynx group ($N = 16$). Genomic DNA from peripheral blood was isolated with the salting out-method.[19] All patients and controls gave their informed consent for their participation in this study. This study was approved by the local ethical committee of the UMC Utrecht (METC protocol #96/267).

HLA typing

HNSCC patients and controls were typed for HLA-A, -B, -C, -DR and -DQ at low resolution by sequence specific priming (SSP) and SSP results were interpreted with the appropriate software according to the manufacturer's protocols (Biotest, Dreieich, Germany). High resolution sequencing based typing (SBT) was performed for HLA-B*40 alleles with the HLA-B High Resolution Typing System (Applied Biosystems, Foster City, CA, USA).

High resolution MICA SBT

MICA alleles were identified by sequencing exons 2–4. A single PCR was performed, amplifying exons 2–5 with the Expand Long Template PCR system (Roche Diagnostics GmbH, Mannheim, Germany). Amplification was performed in 50 μl with final concentrations of 300 nM for both 5′ and 3′ amplification primers[20] (Sigma-Genosys Ltd., Haverhill, United Kingdom), 350 μM of each dNTP (Invitrogen Ltd., Paisley, United Kingdom), 250 ng DNA, 1.0% buffer 1, 1.75 mM $MgCl_2$ and 0.052 units/μl of the thermostable Taq DNA polymerase enzyme mix (included in the Expand Long Template PCR system). The MICA PCR was performed in a 9600 Perkin Elmer thermal cycler (Applied Biosystems). An initial denaturation step at 94 °C for 2 min was followed by 28 cycles of 10 s at 94 °C, 30 s at 62 °C and 2 min at 68 °C. A final extension was performed for 7 min at 68 °C and hold at 4 °C. Subsequently the PCR product was purified with 1 unit of SAP and 10 units of exonuclease I (both from USB Corporation, Cleveland, USA) prior to sequencing.

Sequencing reactions were performed both in forward and reversed directions for exons 2–4 with sequencing primers described earlier.[20] The Big Dye Terminator Ready Reaction Mix v1.1 kit was used for sequencing reactions (Applied Biosystems). MICA was sequenced on an ABI 377 DNA sequencer (Applied Biosystems). Assignment of MICA alleles was performed with software HETERO and ALLELE.[21,22]

Statistical analysis

The allele frequency distribution was evaluated in the control group and compared with the allele frequency distribution in the total patient group, the four tumour location