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Genes in the HLA region indicative for head and neck squamous cell carcinoma

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Abstract

The majority of genes in the HLA region are directly or indirectly involved in immunological functions. They comprise HLA, HLA-related and non-HLA-related genes. Aberrant HLA expression patterns, including heterogeneous and negative HLA expression, are observed in specimens from head and neck squamous cell carcinoma (HNSCC). To explore the possible role of genes in the HLA region other than the classical HLA genes, susceptibility regions within the HLA region for HNSCC were defined in this study. Microsatellite analysis for 49 microsatellites dispersed throughout the HLA region, in combination with the DNA pooling approach of respectively one control DNA pool and three patient DNA pools, based upon the tumour location, offered an efficient method to define susceptibility regions. In the oral cavity three significant susceptibility regions were localized, one in the class I region (330 kb), and two in the class II region (170 and 210 kb). Eighteen genes from these regions were tested for their RNA expression in oral cavity tumour tissue and compared to expression in the surrounding healthy tissue. A significant increased MICA RNA expression in tumour tissues compared to healthy surrounding tissue and a significant decreased HSD17B8 RNA in tumour tissues compared to surrounding healthy tissue, particular in those tumours without lymph node metastasis, were observed. A trend for decreased RXRβ and NOTCH4 RNA expression was observed in tumour tissue. In addition to the classical HLA genes, other genes within the HLA region define susceptibility for oral squamous cell carcinoma of the head and neck.

Keywords: HLA; HNSCC; DNA pools; HSD17B8; MICA; RXRβ

1. Introduction

The human leukocyte antigen (HLA) region on the short arm of chromosome 6 is the most polymorphic region in the human genome. Approximately 250 HLA, HLA-related and non-HLA-related genes are classified as expressed genes and represent about 60% of the total number of genes present in the HLA region. These genes are involved in several biological processes, with emphasis on immunological functions (Horton et al., 2004). Extensive research to identify disease-susceptible genes in the HLA region is an ongoing process (Matsuzaka et al., 2002; Oka et al., 2003; Ota et al., 1999; Smerdel et al., 2002; Zanelli et al., 2001) but remains difficult due to the number of genes present, the complex metabolism and the direct/indirect effects of each gene.

Head and neck squamous cell carcinoma (HNSCC) is a very aggressive tumour from epithelial origin and is located in the upper-aerodigestive tract. The conventional therapies are good and effective for patients diagnosed at an early stage of the disease. In general many patients present an advanced stage of disease at diagnosis, resulting in decreased treatment efficacy and life expectancy (Tromp et al., 2005). For the development of an effective anti-tumour immunotherapeutical treatment it is important to understand the biological mechanisms triggering the development and metastasis of HNSCC.

Expression patterns of the HLA genes in HNSCC showed complex patterns (Koene et al., 2004; Koene, 2005). In addition associations with the HLA-B*40-DRB1*13 haplotype and

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the HLA-B*35 allele in tumour location subgroups were identified (Reinders et al., 2006). Expression analysis of genes involved in the antigen presenting machinery of class I alleles could not target specific loss of HLA expression in HNSCC (Koene, 2005). Although these studies indicate that HLA is associated and expression is affected in HNSCC, genes other than the HLA genes might additionally be involved, resulting in escape from tumour immunosurveillance. For example, HLA-B*44 expression loss was observed in several types of carcinomas that was due to the defective transcription of TAPBP (TAP binding protein, tapasin) (Cabrera et al., 2003, 2005).

Screening of pooled DNA samples of respectively patient and control subjects with microsatellite analysis offers a good and efficient method for identification of disease-susceptibility regions (Collins et al., 2000). Pooling of DNA samples involves combining equal amounts of DNA from controls and patients in respectively a control and a patient pool to efficiently analyse large sample numbers. In the past microsatellite markers, dispersed throughout the HLA region, have extensively been characterized (Foissac et al., 1997, 2000; Gourraud et al., 2004; Matsuzaka et al., 2000, 2001; Tamiya et al., 1998, 1999). Microsatellite markers are helpful in the definition of disease-associated regions. Defining an associated region requires the testing of many microsatellite markers and with the use of DNA pools this is relatively easy. This approach has for example been applied to localize a non-melanoma skin cancer susceptibility region within the major histocompatibility complex (Oka et al., 2003) and to detect linkage disequilibrium of polymorphic restriction fragments in insulin-dependent mellitus (Arnheim et al., 1985).

We investigated whether we could identify disease-susceptibility regions within the HLA region with HNSCC and their location using microsatellite analysis and DNA pooling. Genes of interest were subsequently studied for their RNA expression, with the quantitative PCR (Q-PCR) approach, in oral cavity tumour tissue compared to surrounding healthy tissue.

2. Material and methods

2.1. Samples

In the period 1996–2002 HNSCC patients ($N = 346$), with and without lymph node metastasis, were collected at the University Medical Centre Utrecht. Patients were grouped according to the location of their tumour: larynx ($N = 101$), oral cavity ($N = 159$) and oropharynx ($N = 86$). Healthy volunteers ($N = 106$) were used as controls in this study. Informed consent for this study was obtained from all patients and controls participating. This study was approved by the ethical committee of the UMC-U (METC protocol #96/267).

2.2. DNA pools

DNA used for the generation of the DNA pools was isolated from peripheral blood from patient and control samples with the salting-out method (Miller et al., 1988). Four different DNA pools were generated, one control DNA pool, and three patient DNA pools based upon the tumour location. Pooled DNA was prepared as described (Collins et al., 2000). An accurate DNA concentration measurement was performed with the PicoGreen method (Molecular Probes, Eugene, Oregon, USA). Equal amounts of DNA for each individual were pooled and the DNA concentration of the pooled DNA were adjusted to 2.4 ng/μl for the control and oral cavity and 2.0 ng/μl for the larynx and the oropharynx DNA pool with a solution of 10 mM Tris and 0.1 mM EDTA.

2.3. Microsatellite analysis

The four DNA pools were tested with 49 polymorphic microsatellite markers: 30 markers were located in the HLA class I region, 16 in the class II region and three in the class III region (Foissac et al., 1997, 2000; Gourraud et al., 2004; Matsuzaka et al., 2000, 2001; Tamiya et al., 1998, 1999). For all the microsatellite markers tested the same PCR reagents and conditions were used. Final concentrations of the reagents in the PCR mixture were: 1.2 ng/μl of pooled DNA, 1× PCR buffer (1.5 mM $MgCl_2$) (Applied Biosystems Co., Japan), 200 nM of each dNTP (Applied Biosystems), 0.025 U/μl AmpliTaq Gold DNA polymerase (Applied Biosystems) and 1 μM of each of the amplification primers, the 5′ labelled with 6-carboxyfluorescein amidite (6-FAM) (Proligo Japan K.K. Science Center, Kyoto, Japan), in a total volume of 20 μl. Amplification was initiated by a hot-start at 95 °C for 9 min, followed by 1 min at 57 °C and 1 min at 72 °C and amplification was performed in 40 cycles of 45 s at 96 °C, 45 s at 57 °C and 1 min at 72 °C and ending with hold at 4 °C. PCR products were subsequently 1:20 diluted with MilliQ, vacuum dried, mixed with formamide and the size standard marker GS500 ROX (Applied Biosystems) and denatured for 3 min at 95 °C. PCR products were analysed on an ABI PRISM 3700 sequencer (Applied Biosystems). Allele length determination for each microsatellite marker was performed with GeneScan software (Applied Biosystems).

DNA pools were validated by genotyping the markers D6SC1_4_1 and D6SC5_3_1, both located in the class I region, in the DNA pools and the individual samples with the same protocol as described above. For the individual samples 10 ng of DNA was used in the PCR mixture. Subsequently the allele frequencies observed in the DNA pools were compared to the allele frequencies observed in the individual samples. Upon validation, all 49 microsatellites were tested in the four DNA pools. Allele frequencies for these microsatellite markers were determined by calculating the relative value of each peak height for an allele relative to the sum of all peak heights of all alleles of a particular microsatellite marker (Collins et al., 2000). For identification of disease-susceptibility regions the allele frequency distribution for each microsatellite marker was determined of the control DNA pool with each of the patient DNA pools, performing a multi χ^2 analysis and in addition Fisher's exact test for the individual alleles (GraphPad InStat Software, San Diego, California, USA).