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ORIGINAL ARTICLE

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Multiple mechanisms generate HLA class I altered phenotypes in laryngeal carcinomas: high frequency of HLA haplotype loss associated with loss of heterozygosity in chromosome region 6p21

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Abstract Major histocompatibility complex (MHC) class I loss or downregulation in cancer cells is a major immune escape route used by a large variety of human tumors to evade anti-tumor immune responses mediated by cytotoxic T lymphocytes. Multiple mechanisms are responsible for such HLA class I alterations. However, the precise frequency of these molecular defects has not been clearly determined in tumors derived from specific tissues. To analyze such defects we aim to define the major HLA class I-altered phenotypes in different tumor types. In this paper we report on HLA class I expression in 70 laryngeal carcinomas. We used immunohistological techniques with a highly selective panel of anti-HLA monoclonal antibodies (mAb), and polymerase chain reaction (PCR) microsatellite amplification of previously selected microsatellite markers (STR) located in chromosome 6 and 15. DNA was obtained from microdissected tumor tissues and surrounding stroma to define the loss of heterozygosity (LOH) associated with chromosome 6p21. Our results showed that LOH in chromosome 6 produced HLA haplotype loss (phenotype II) in 36% of the tumors. In addition, HLA class I total loss (phenotype I) was found in 11%; HLA A or B locus downregulation (phenotype III) was detected in 20%; and HLA class I allelic loss (phenotype IV) in 10% of all cases. We sometimes observed two or more associated mechanisms in the same HLA-altered phenotype, such as LOH and HLA total loss in phenotype I. In only 23% of tumors it was not possible to identify any HLA class I alteration. We conclude that the combination of immunohistological techniques and molecular analysis of tumor DNA obtained from microdissected tumor tissues provides a means for the first time of determining the actual frequency of the major HLA class I-altered phenotypes in laryngeal carcinomas.

Keywords Escape · HLA · Laryngeal carcinoma · LOH

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Introduction

Tumor cells frequently undergo major histocompatibility complex (MHC) class I molecule loss during tumor development [14, 18]. These HLA losses produce tumor cells that are able to escape anti-tumor T cell immune responses. Defects in the antigen processing machinery and in HLA class I antigens in malignant cells may have a significant impact on the clinical course of malignant diseases and on the outcome of T cell-based immunotherapy. The presence of these altered MHC phenotypes is probably a consequence of T cell immunoselection of MHC class I-deficient tumor variants, as demonstrated by the finding of MHC class I-positive phenotypes in metastatic tumor clones produced in immunodeficient nude/nude athymic mice after the injection of MHC class I-negative tumor clones [16].

Multiple molecular mechanisms have been determined as responsible for MHC alterations [4, 26]. One example is the occurrence of structural defects in one copy of the β_2-microglobulin gene associated with loss of the wild-type β_2m allele due to a mitotic recombination event or to the total or partial loss of chromosome 15 (phenotype I, HLA total loss) [30]. These mutations might include base-pair (bp) substitutions and deletions which range from the loss of one bp to the loss of extensive segments of the gene [2, 41]. Downregulation of the TAP gene appears to be caused in most reported tumors by defective mechanisms of gene regulation, and can therefore be corrected by interferon-gamma (IFN-γ)

[34]. Loss of one HLA haplotype, which has been associated with loss of heterozygosity in chromosome 6p21, has been described in different tumors and cell lines of different origin (e.g. melanoma, cervix carcinoma, laryngeal carcinoma, colon carcinoma, pancreatic carcinoma; phenotype II, HLA haplotype loss) [24, 37]. This phenotype is caused by the loss of variable portions of genomic DNA due to chromosomal segregation, nondysjunction or mitotic recombination in the short arm of chromosome 6 [13]. HLA class I allele-specific loss, a frequent modification, is caused by mutations in the genes encoding the HLA class I heavy chain (phenotype IV) [28, 35]. These mutations include chromosomal breakpoint, bp insertions and bp substitutions in exons or introns [5, 22, 26]. However, the precise frequency of particular mechanisms in different tumors tissues is not yet known. This information is of particular relevance when a cancer patient enters a T-cell-based immunotherapy trial.

Our laboratory is engaged in a long-term research project to determine the actual frequency of the different mechanisms that lead to HLA class I-altered phenotypes in human tumors. To this end, we have developed a laboratory approach that allows us to obtain DNA and RNA after tissue microdissection of cryopreserved tumor tissue sections [32]. This has made it possible to define loss of heterozygosity (LOH) in chromosome 6p21 using a selected panel of microsatellite markers (STR) located in chromosome 6 in or near the HLA region in a variety of samples from different tumors [23, 31] and has shown that LOH is a widespread mechanism used by many tumor cells to produce HLA class I-deficient tumor variants [18].

The present paper reports the actual frequencies of different HLA class I tumor phenotypes in a series of 70 laryngeal carcinomas.

Materials and methods

Patients and tumor specimens

Seventy laryngeal carcinoma tissue samples were obtained from patients treated at the Department of Otolaryngology (Hospital Universitario Virgen de las Nieves in Granada, Spain) after previous informed consent and approval of the protocol by the institutional review board. Tumor samples were snap-frozen in isopentane cooled with liquid nitrogen and stored in liquid nitrogen until sectioned for study.

HLA typing of peripheral blood lymphocytes and tumor tissues

Peripheral blood lymphocytes (PBL) were isolated from cancer patients and HLA-typed in our laboratory using the standard complement-dependent microcytotoxicity test.

Immunohistological techniques for cryostatic tumor sections were used to determine the HLA phenotype, with a biotin–streptavidin-amplified (B–SA) detection system (supersensitive Multilink-HRP/DAB; BioGenex). The panel of antibodies and system used to score the results have been described previously by Cabrera et al. [6].

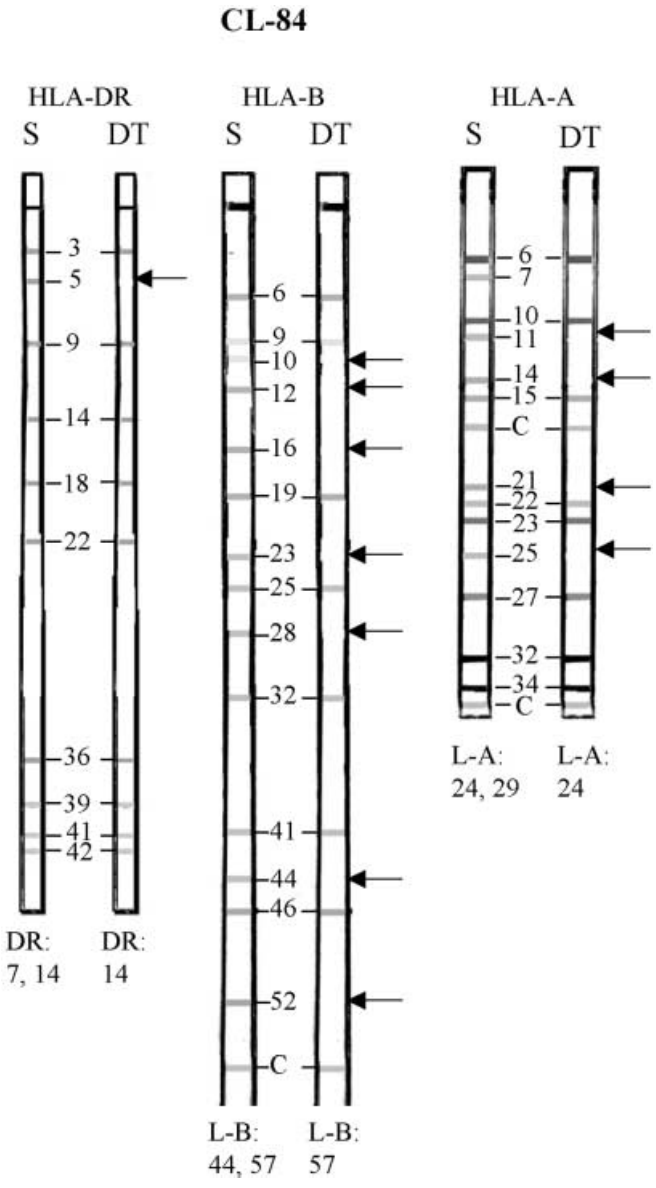

Fig. 1. HLA haplotype loss in laryngeal carcinoma CL-84 as detected by SSO analysis of HLA genes. In comparison to the autologous microdissected stroma (*S*), the microdissected tumor (*DT*) lost HLA A29, B44 and DR7

Microdissection, DNA isolation, LOH analysis and HLA tumor typing

Cryopreserved tissue sections between 4–8 μm thick, stained with a 0.05% wt/vol solution of toluidine blue, were used for microdissection according to the procedure described by Ramal et al. [32]. DNA from the microdissected fragments (tumor and stroma) was obtained with a DNA isolation kit (Qiamp Tissue Kit; Qiagen, Wetsburg, Leusden, the Netherlands).

DNA was studied with 8 STR markers that mapped chromosome 6, and with 2 markers for chromosome 15. Eight of the markers (D6s311, D6s291, D6s273, D6s265, D6s105, D6s276, D15s209 and D15s126) have been described previously by Ramal et al. [31]. In the present study, 2 markers next to loci B-C.1.2.c and C.1.2.5-were added [36]. Polymerase chain reaction (PCR), electrophoresis and data analysis were as previously described [31]. Loss of heterozygosity was considered to exist when the signal of

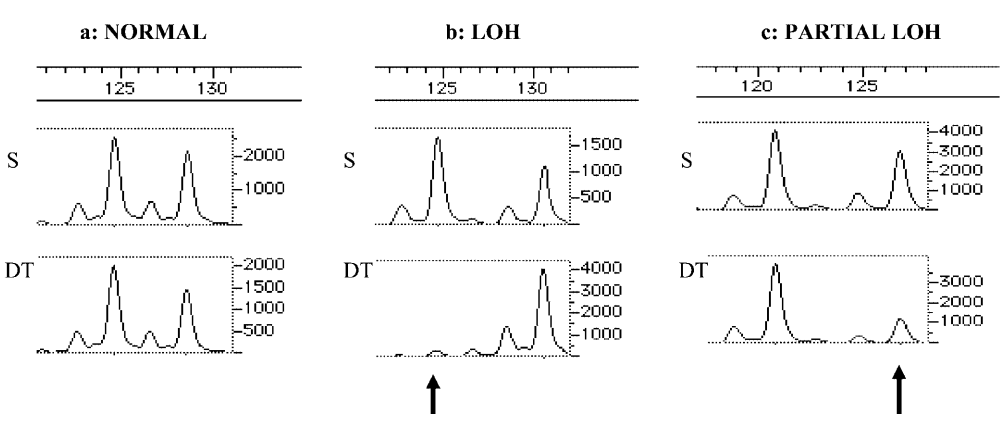

Fig. 2A–C. Microsatellite PCR amplification of the D6S265 marker in DNA obtained from microdissected laryngeal tumor tissue (*DT*) and microdissected autologous stroma (*S*). Three different patterns are shown: **A** normal pattern with no differences in the amplification signal or in the electrophoretic mobility between tumor and stroma DNA; **B** clear LOH in microdissected tumor DNA as compared to autologous stroma; and **C** partial LOH (>25% reduction in signal intensity)

Table 1. Laryngeal tumors with defined HLA haplotype loss in chromosome 6

Tumor	HLA class I tumor phenotype by immunohistology						HLA Haplotype loss	6q	6p					
								311	291	273	C.1.2c	C.1.2.5	265	105	276
No alterations detected															
CL57	A11	A29	B35	B60		Bw6	A11/B40/DR♦	N	H	H	N	L	L	L	L
CL66	A69	A33	B8	B14		Bw6	A33/B14/DR1	L	L	L	L	L	L	L	H
CL76	A24	-	B13	B51	Bw4		A24/B51/DR3	L	H	L	L	L	H	H	H
Phenotype I															
CL15	A2	-	B35	B50		Bw6	A2/B35/DR♦	L	H	L	H	H	L	N	H
CL27	A2	A23	B44	B14	Bw4	Bw6	A23/B44/DR4	N	H	H	L	H	L	L	L
CL79	A24	A11	B18	B51	Bw4	Bw6	A24/B18/DR11	N	L	L	L	L	L	L	N
Phenotype II															
CL47	A3	A31	B7	B27	Bw4	Bw6	A31/B27/DR4	H	L	L	H	L	L	L	H
CL62	A1	A30	B52	B62	Bw4	Bw6	A30/B62/DR14	L	H	L	L	L	H	H	L
CL89	A2	A26	B18	B38	Bw4	Bw6	A2/B18/DR1	L	L	H	L	L	L	H	L
Phenotype III															
CL34	A1	A2	B7	B51	Bw4	Bw6	A2/B7/DR1	N	L	H	L	L	L	H	L
CL61	A3	-	B8	B44	Bw4	Bw6	A3/B44/DR♦	N	H	N	L	L	H	L	H
CL41	A24	A25	B35	B18	Bw4	Bw6	A25/B18/DR15	N	L	L	L	L	L	L	L
CL42	A2*	A28*	B51	B44	Bw4		A2/B44/DR7	H	L	H	L	L	L	L	L
CL68	A2	A33	B51	B65	Bw4	Bw6	A33/B65/DR13	H	H	L	H	H	L	H	H
CL48	A2	A11	B51	B49	Bw4		A11/B51/DR4	H	H	H	H	H	H	H	H
CL82	A2	A25	B7	B35		Bw6	A2/B7/DR3	H	N	H	H	H	L	L	H
CL83	A29	A30	B13	B57	Bw4		A30 /B13/DR7	N	H	L	H	H	L	L	L
Phenotype IV															
CL49	A24	A66	B35	B41		Bw6	A66/B41/DR7	L	L	L	L	L	H	L	L
CL81	A1	A2	B8	B49	Bw4	Bw6	A1/B8/DR3	L	L	L	H	L	L	L	L
CL84	A24	A29	B44	B57	Bw4		A29/B44/DR7	N	L	H	L	H	L	L	L
CL91	A29	A30	B44	B18	Bw4	Bw6	A29/B44/DR7	L	L	L	L	L	L	H	L
CL64	A2	A24	B18	B60		Bw6	A2/B40/DR14	L	L	L	H	H	L	L	H
CL95	A11	A69	B40	B35		Bw6	A69/B35/DR1	N	L	H	H	L	L	L	N
CL92	A24	A33	B13	B14	Bw4	Bw6	A33/B14/DR♦	N	N	H	L	L	H	H	L

White boxes indicate positive reaction with the corresponding anti-HLA class I mAb. Black boxes indicate negative reaction. Alleles without boxes indicate absence of specific mAbs
L= LOH; N= Normal, absence of alterations; H= Homozygous

♦ These cases showed no DR loss
* The mAbs used did not distinguish between these two alleles

Table 2. Laryngeal tumors with LOH in chromosome 6 (HLA haplotype loss not defined)

Tumor	HLA class I tumor phenotype by immunohistology						6q	6p						
							311	291	273	C.1.2.c	C.1.2.5	265	105	276
No alterations detected														
CL39	A2	A11	B39	B63	Bw4	Bw6	N	L	H	L	L	L	L	N
CL43	A2	A24	B63	B35	Bw4	Bw6	H	L	L	L	H	H	L	L
CL63	A30	A66	B18	B52	Bw4	Bw6	H	L	L	L	L	H	H	L
CL74	A24	-	B62	B39		Bw6	H	L	N	L	L	H	H	H
CL75	A11	A29	B45	B60		Bw6	N	N	H	N	L	L	L	H
CL77	A30	-	B14	-		Bw6	N	H	H	L	H	L	L	L
CL93	A2	-	B38	B41	Bw4	Bw6	L	H	L	H	L	N	N	L
CL103	A3	A11	B27	-	Bw4		N	N	H	H	H	L	N	L
Phenotype III														
CL46	A2	A1	B51	B57	Bw4	Bw6	L	H	L	L	L	L	L	N
CL94	A2	A24	B65	B18		Bw6	L	H	L	L	L	N	L	L
Phenotype IV														
CL72	A2	A24	B44	B50	Bw4	Bw6	L	N	L	H	H	L	L	N
CL73	A2	A26	B44	B35	Bw4	Bw6	H	H	L	H	H	H	H	L
CL88	A2	A3	B7	B50		Bw6	H	L	L	H	L	N	L	H
CL97	A29	A68	B14	-		Bw6	N	H	L	L	L	N	H	L

White boxes indicate positive reaction with the corresponding anti-HLA class I mAb. Black boxes indicate negative reaction. No boxes indicate absence of anti-HLA class I mAb.

L= LOH equal to or greater than 0.25% reduction as compared with the autologous control. The HLA haplotype lost could not be determined due to the heterogeneity of the tumor sample.
N= Normal, absence of alterations
H= Homozygous

one allele was reduced by more than 25% in the tumor sample compared to the control stroma sample. Haplotype loss was considered to exist when a tumor showed a reduction in three or more STR markers.

To determine the type of HLA haplotype loss, sequence-specific oligonucleotide (SSO) analysis was performed using Dynal Reli HLA-A, B, DR, and DQ with DNA obtained from stroma and tumor cells of samples that showed LOH of 6p21.

Results

LOH analysis and HLA genomic typing

Loss of heterozygosity in the 6p21.3 region was analyzed with 7 previously selected microsatellite markers [31]. DS6276 was the most telomeric and DS291 the most centromeric. According to the criteria described in Materials and methods, LOH was found in the 6p21.3 region in 38 cases (54%) of laryngeal carcinoma.

In most cases with LOH for 6p21.3 (24 out of 38), the missing HLA haplotype specificities could be defined during genomic HLA typing. In these cases there was no residual signal in the peak that defined the missing STR allele (Figs. 1 and 2B).

Table 1 shows the relationships between the HLA phenotypes identified by immunohistological techniques, the HLA haplotype losses, and the results of STR analysis for 6p21.3 and 6q for 24 cases of laryngeal carcinoma. Phenotype and genotype correlated well because most of the allelic losses we detected were included within the haplotype losses. In three cases (CL-47, CL-62 and CL-89) phenotype II, detected by immunohistological methods, corresponded to the haplotype lost. In 7 cases phenotype IV would have been classified as phenotype II if we had been able to analyze all alleles; however, the allele identified by immunohistochemical technique as having been lost was included within the HLA haplotype lost. In three cases (CL-57, CL-66 and CL-76) with LOH for HLA, the immunohistochemical technique revealed no alterations because the alleles of interest belonged to the retained haplotype, and moreover were homozygotic for HLA-Bw4 or HLA-Bw6. These cases were classified as phenotype II on the basis of molecular analysis. In other cases in this group, LOH for HLA was accompanied by loss of expression of the other haplotype, which led to phenotype I. When haplotype loss was accompanied by downregulation of the A or B locus the tumor was classified as phenotype III.

Table 3. Laryngeal tumors without LOH in chromosome 6

Tumor	HLA class I tumor typing by immunohistology					
No alterations detected						
CL23	A26	A30	B18	B38	Bw4	Bw6
CL35	A2	A30	B13	B27	Bw4	
CL37	A29	-	B44	B7	Bw4	Bw6
CL55	A31	A34	B37	B41	Bw4	Bw6
CL58	A11	-	B18	-		Bw6
CL65	A69	A33	B14	B22		Bw6
CL67	A2	A24	B62	B39		Bw6
CL70	A24	A30	B7	B50		Bw6
CL71	A3	-	B51	B38	Bw4	
CL78	A2	-	B35	B61		Bw6
CL85	A24	A11	B7	B35		Bw6
CL96	A1	A2	B13	B62	Bw4	Bw6
CL98	A1	A11	B8	B62		Bw6
CL99	A24	A29	B61	-		Bw6
CL100	A3	-	B7	B35		Bw6
CL102	A25	A30	B18	-		Bw6
Phenotype I						
CL36	A2	A24	B27	B35	Bw4	Bw6
CL50	A24	A25	B27	B51	Bw4	
CL56	A1	A24	B8	B17	Bw4	Bw6
CL80	A2	A3	B7	B27	Bw4	Bw6
CL101	A2	A24	B18	B35		Bw6
Phenotype III						
CL40	A30	A33	B14	B18		Bw6
CL51	A26	A30	B13	B40	Bw4	Bw6
CL59	A11	A32	B35	B53	Bw4	Bw6
CL90	A29	-	B7	B44	Bw4	Bw6
Phenotype IV						
CL22	A2	A29	B44	B18	Bw4	Bw6
CL38	A2	A30	B14	B18		Bw6
CL53	A28	A29	B44	B14	Bw4	Bw6
CL54	A29	A33	B44	B14	Bw4	Bw6
CL86	A2	A26	B44	B14	Bw4	Bw6
CL87	A3	A25	B7	B44	Bw4	Bw6
CL104	A2	-	B44	B51	Bw4	

Table 2 shows the findings in the 14 remaining cases with LOH for 6p21.3, in which SSO was unable to identify which haplotype was lost. In these cases a residual peak showed lower-than-normal values for the allele (Fig. 2C). In this group, immunohistochemical methods failed to identify any alterations in 8 cases, whereas 2 cases were classified as phenotype III and 4 as phenotype IV. Samples that showed no alterations on immunohistochemical testing and samples classified as phenotype IV were counted as phenotype II in the final analysis.

Table 3 shows the phenotypes for the 32 cases which did not have LOH for 6p21.3 or 6q. In 16 cases no phenotypic alterations were found; 5 cases were classified as phenotype I, 4 as phenotype III and 7 as phenotype IV.

Frequency distribution of phenotypes with altered HLA expression

Immunohistological studies with mAb and molecular analyses with STR of the 6p21.3 region showed the most frequent HLA phenotype in the laryngeal carcinomas we studied to be phenotype II, which accounted for 25 cases (36%) (Fig. 3). This group included tumors with LOH in the 6p21.3 region, and which were previously classified by immunohistological techniques as phenotype II or IV or as phenotypically normal, in view of the fact that were unable to determine which HLA allele was lost. The second most frequent was phenotype III, which accounted for 14 cases (20%), 10 of which (14%) had LOH in 6p21.3. The third most frequent was phenotype I, which accounted for 8 cases (11%), 3 of which (4%) showed LOH. Phenotype IV was identified in only 7 cases (10%). In the remaining 23% of the cases we detected no phenotypic alterations.

β_2-microglobulin gene analysis

Two STR markers that flanked the β_2-microglobulin gene (DS15209, centromeric and DS15126, telomeric) revealed LOH for at least 1 marker in 29/70 (41%) of the laryngeal carcinomas we analyzed (Table 4). Five tumors had no HLA alterations, 6 had LOH in 6p with a normal phenotype, 5 had phenotype I (2 with LOH in 6p) 1 case showed phenotype II, 5 had phenotype III (3 with LOH in 6p) and 8 had phenotype IV (3 with LOH in 6p).

Discussion

Monoclonal antibodies that specifically define HLA class I alleles in solid tissues make it possible to define the precise alteration in the expression of these molecule using immunohistological techniques [7, 18]. Nevertheless, there are still a number of important HLA allele specificities that cannot be defined immunohistologically due to the lack of the appropriate anti-HLA mAb capable of maintaining their specific reactivity in cryostatic tissue sections. This means that in some cases it is not possible to define particular HLA class I alterations or – more importantly – to distinguish between different altered HLA phenotypes such as HLA allelic loss or HLA haplotype loss (phenotypes IV and II) [7].

It is already well established that multiple mechanisms can generate HLA class I-deficient tumor variants [4, 26]. However, some mechanisms are more frequent in tumors derived from certain tissues than in other types

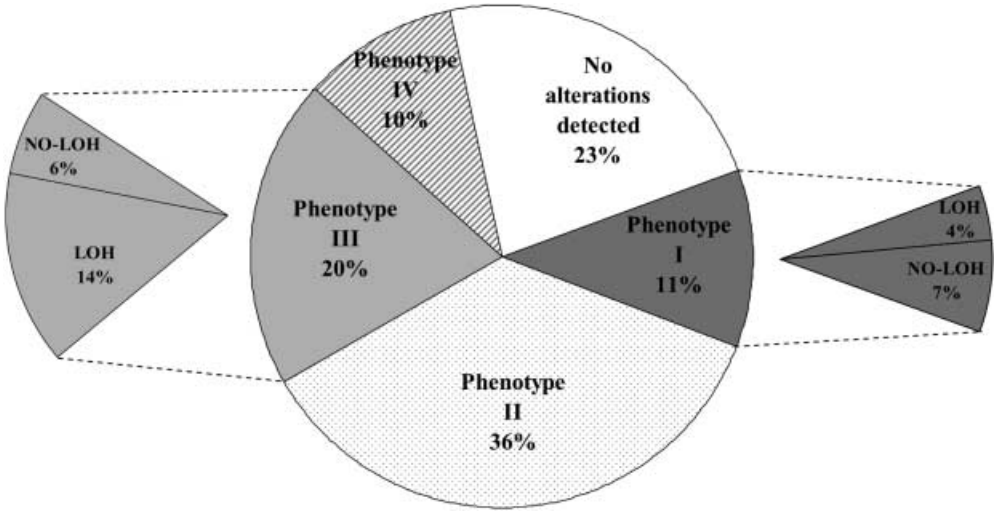

Fig. 3. Distribution of the percentages of the four major HLA class I phenotypes in laryngeal carcinomas: HLA class I total loss (phenotype I); HLA class I haplotype loss (phenotype II); HLA class I A and B locus loss (phenotype III) and HLA class I allelic loss (phenotype IV)

Table 4. LOH for β_2m

Tumor	D15s126	D15s209	LOH 6p21.3
No alterations detected by immunohistology			
CL35	N	L	N
CL43	0.5	H	L
CL58	0.7	0.7	N
CL63	H	0.2	L
CL66	H	L	L
CL67	0.6	0.6	N
CL74	0.7	0.7	L
CL76	L	L	L
CL93	N	0.6	L
CL96	0.7	N	N
CL103	0.7	N	L
Phenotype I			
CL15	0.7	0.7	L
CL51	0.7	0.7	N
CL79	H	0.4	L
CL80	H	0.4	N
CL101	H	L	N
Phenotype II			
CL89	H	L	L
Phenotype III			
CL82	0.7	0.6	L
CL92	H	0.5	L
CL38	L	H	N
CL68	H	L	L
CL40	0.4	0.4	N
Phenotype IV			
CL22	H	0.7	N
CL53	0.7	0.7	N
CL54	H	L	N
CL72	H	L	L
CL86	H	0.7	N
CL88	H	0.7	L
CL91	0.7	0.7	L

N = no LOH; L = LOH; H = homozygous

of tumor. For instance, β_2m mutations are found in melanomas and colon carcinomas, where they give rise to HLA class I total loss [17, 30]. In contrast, we have not been able to identify to date similar mutations in HLA class I total loss phenotypes found in bladder or in laryngeal carcinomas (unpublished results). These preliminary data may indicate that some molecular mechanisms might be more frequent than others in tumors derived from different tissues. In this context, we have been able to determine the frequency of HLA haplotype loss associated with LOH in chromosome region 6p21 in laryngeal carcinomas. This was possible thanks to a combined phenotypic and molecular analysis applied to samples obtained from microdissected tumor tissues. Our approach reduced from 39% to 23% the number of cases in which it was not possible to detect HLA class I alterations using immunohistological methods only. This reduction was achieved as a result of the increased detection of LOH associated with 6p21.3 in cases with an apparently normal phenotype. It was also possible to identify samples with both LOH in one HLA haplotype and lack of expression of HLA specificities in the other, giving rise to tumor samples with no HLA expression (phenotype I) or a combination of LOH and downregulation of HLA-A or B locus products (phenotype IIIa or IIIb).

The frequency of LOH for 6p21.3 as defined in this study is the highest reported to date for laryngeal carcinomas (54%). This figure comprises 36% of the samples with HLA haplotype loss, 4% with HLA total loss and 14% in HLA-A or B locus-specific downregulation (see Fig. 1). This may reflect the fact that in previous studies we did not use microdissected tissues, hence the contaminating stroma may have masked the LOH [13]. It is also possible that the number of STR markers was too small, or the established cut-off level too high, to accurately identify LOH [15, 23].

On the basis of genomic HLA typing of cases with LOH for 6p21.3, we were able to identify two types of laryngeal carcinoma. One type showed a complete pattern of LOH for 6p21.3 and hemizygosity for genomic HLA, and probably corresponded to a clonal population of tumor cells. The other fulfilled the criterion for LOH (reduction of $>25\%$), but showed no alterations in genomic HLA; this second type probably consisted of

a heterogeneous population of tumor cells with positive and negative tumor cells for HLA class I antigens.

Loss of heterozygosity for different chromosome regions has been reported in head and neck tumors [11]. Tumor progression seems to be associated with the loss of genetic chromosomic material. The earliest alterations described in premalignant lesions are LOH for 9p21, 17p13, 18q21 and 3p [39]; these are regions that contain tumor suppressor genes [25, 29]. The transition of hyperplastic to in situ laryngeal carcinoma seems to be associated with LOH for 11q13, 13q21 and 14q24. Similarly, progression from carcinoma in situ to invasive carcinoma requires LOH for 6p21.3, 8 and 4q26–28 [8].

It is also well established that tumor antigens can originate in the early stages of laryngeal carcinoma [38]. Examples are mutations in P53 [3], enhanced expression of proto-oncogenes such as D1 cyclin [9], or growth factors such as EGF [21], and neoexpression of the MAGE gene family antigens [12]. The absence from a given HLA haplotype of a set of HLA antigens required for antigen presentation to cytotoxic T lymphocytes no doubt favors the immune escape of a particular tumor clone with these phenotypic characteristics during the transition from carcinoma in situ to invasive carcinoma.

The loss of a particular HLA haplotype also implies the elimination of MICA and MICB alleles. It was recently established that these molecules are the ligands of activatory natural killer (NK) receptors [1], and are also present in $CD8^+$ lymphocytes [19] and gamma–delta intraepithelial-positive T cells [20]. In experimental murine tumors the ectopic expression of the RAE-1 protein - the homolog of the MIC family - activates NK cells to kill HLA class I-positive tumor cells [10, 27]. Therefore, in HLA haplotype-deficient tumor cells the capacity to activate immune cells under stressful situations is impaired by the absence of MIC-A and B genes.

The retention of a given HLA haplotype and loss of another is a marker of clonality in a large percentage (36%) of laryngeal tumors. This characteristic may result from a process of immunoselection that eliminates cells that express the immunodominant HLA haplotype for tumor peptide presentation. As a result, the tumor clone that has lost this haplotype would escape immune system control. The retained HLA haplotype would be an inefficient presenter of tumor antigens, and thus would not be eliminated [33].

Different T cell-based immunization protocols have been developed in the last few years to treat human cancers with peptides specifically presented by HLA class I molecules [40]. Our results are of potential use to clinical oncologists, as they may make it easier to identify and select tumor peptides presented by a particular HLA class I molecule that is not lost during tumor progression.

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