

Genetic alterations of HLA-class II in ovarian cancer

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The immune system controls tumor formation through identification and elimination of cellular alterations. Consequently, cancer development in immune competent hosts depends on strategies to evade the immune system. Modulation of tumor antigen-specific immune responses by aberrant expression of HLA-class I and II molecules is well documented in a variety of carcinomas including ovarian cancer. To date, little data are available about molecular mechanisms responsible for altered HLA-class II phenotypes in tumors. In our sample of 10 Caucasian patients with ovarian carcinoma, a semiquantitative analysis was performed for HLA-class II loci DRB1 and DQB1 in malignant and normal ovarian tissue. Gene amplifications were identified in 62.5% of analyzed alleles and deletions in 17.5%, demonstrating that genomic aberrations of 6p21.3 are common and that copy number gain is more frequent than loss. Moreover, amplifications are most pronounced in advanced-stage tumors. To evaluate genotype-phenotype relation, immunohistochemical analyses were performed and revealed *de novo* expression of HLA-class II in 30% of tumors with an inverse association between antigen level and HLA copy number. It remains to be elucidated whether the profound changes of the latter quantities are the result of the host's immunological self-defense, indicate the presence of an oncogene located within the MHC-complex or merely reflect the increasing loss of differentiation of the tumor tissue.

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The concept of tumor immunology is based on the assumption that the immune system is able to discriminate between normal and malignant tissues and to protect the host from tumor development by recognition and subsequent elimination of aberrant cells.¹ The major histocompatibility complex (MHC) and more specifically the human leukocyte antigen (HLA) system is considered to be important in tumor surveillance, since it modulates the efficacy of cytotoxic immune responses through the presentation of tumor antigens. However, malignant cells develop strategies that help to evade autoprotective immunologic mechanisms, thus favoring tumor tolerance and subsequent tumor growth.^{2,3}

In ovarian carcinomas, tumor infiltrating lymphocytes (TILs) have been identified indicating a local immune reaction, and their presence is a marker of better prognosis.^{4,5} Because of a positive correlation between the existence of TILs and the amount of HLA-class II molecules on tumor cells, it is believed that HLA-class II antigens influence the interaction between ovarian cancer and the host's immune response.^{6,7}

Expression of HLA-class II molecules is usually not present in normal ovarian surface epithelium but can be detected in benign, borderline and malignant epithelial ovarian tumors.^{8,9} Different mechanisms may account for *de novo* expression on cancer cells, such as lymphocyte infiltration or tumor-specific pathways.^{10–12} It has been proposed that HLA-class II expression on tumor cells is of biological significance because the introduction of MHC-class II proteins into murine cancer cell lines increases cellular immunogenicity and allows CD4 T-cells to generate effective and long-lasting immune responses.¹³

Apart from the antigen expression pattern, HLA-linked genetic components are supposed to play a role in cancerogenesis as certain alleles or polymorphisms of genes located in the MHC-region have been shown to contribute to cancer susceptibility.^{14–17}

Based on our own previous findings of significant associations of HLA-class II haplotypes DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*1001-DQA1*0101-DQB1*0501 with an increased risk of ovarian cancer,¹⁸ we sought to further investigate the involvement of HLA-class II genes in the pathogenesis of this disease. We performed a comparative semiquantitative analysis evaluating possible gene amplification or deletion mechanisms of HLA-class II genes in ovarian cancer and correlated the results with expression patterns of HLA-class II antigens in tumor tissue.

Material and methods

Patients and specimens

Tissue samples of primary ovarian adenocarcinoma were obtained from 10 therapy-naïve Caucasian women at the time of surgical treatment. Normal ovarian tissue sections and 10 ml of EDTA-anticoagulated blood were additionally collected from every patient. The median age of the patients at initial diagnosis was 50 years (range 37–71 years). They had no signs of other HLA-associated diseases. One patient had Stage I, 3 patients had Stage II and 6 patients had Stage III disease according to the classification of the International Federation of Gynecology and Obstetrics (FIGO).¹⁹ The Ethics committee of the University of Bonn approved the study protocol and all patients gave informed consent before participation in the study.

DNA extraction

DNA was extracted from EDTA-anticoagulated blood by a modified salting-out procedure.²⁰ DNA from formalin-fixed and paraffin-embedded tissue samples was obtained with the QIAamp tissue kit[®] (Qiagen, Hilden, Germany). For this purpose, adjacent tumor sections with more than 70% cancer cells were isolated by microdissection. Control DNA was gained from tissue blocks that were completely free from tumor cells. Per patient, 10 serial slices of 10 µm thickness of tumor and normal ovarian tissue were cut. DNA concentrations of both tissue types were measured and aligned.

Genotyping for HLA-class II

For each HLA-class II locus (DRB1, DQA1, DQB1) alleles were determined in DNA from peripheral lymphocytes and verified in ovarian tissue sections. HLA-genotyping was performed

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with a PCR-based method using sequence-specific primers (PCR-SSP) as published previously.^{18,21}

Comparative semiquantitative analysis

Comparative semiquantitative analysis was performed for the individual HLA-DRB1 and -DQB1 alleles identified by PCR-SSP typing.

To narrow down the extension of chromosomal changes on chromosome 6, the HLA-genes HLA-G and -DPB1, which flank the HLA-class II region, were additionally investigated in 5 patients. HLA-G is a nonclassical HLA-class I gene located closer to the telomere than HLA-DQ and -DR, whereas HLA-DPB1 is a HLA-class II locus which lies closer to the centromere.²² Amplification of exon 2 of the HLA-G gene (356 bp) was performed as reported previously.²³ Amplification of exon 2 of the HLA-DPB1 gene (432 bp) was carried out as described earlier using generic primers (5'-GAGAGTGGCGCCTCCGCTC-3', 5'-TGAATCCCCAACCCAAAGTCCCC-3').²⁴

Because the human factor V (F5) gene on chromosome 1q23 is not involved in tumor dependent changes of gene content in ovarian cancer, exon 10 served as a reference gene for the analysis of quantitative alterations of the HLA-genes of interest.²⁵ The use of F5 allowed to monitor the starting amount of DNA in the PCR and to control for amplification efficacy. Furthermore, comparable amplification efficiencies of the F5 locus in control and tumor tissue excluded polyploidy of the tumor cells. DNA (215 bp) was amplified previously using generic primers (5'-ATCGCCTCTGGGCTAATAGG-3', 5'-TGTTATCACACTGGT GCTAA-3').^{26,27}

In addition to the F5 locus, we included a second standard. The locus DYS205 (134 bp) was amplified from DNA samples of male individuals in a separate PCR as reported earlier.²⁸ A constant amount of PCR product was added as external control to each gel run. This procedure allowed an estimation of gel-dependent variations of DNA-detection.

To ensure comparability of quantitative results, we terminated individual PCRs in the exponential phase of amplification (DRB1/DQB1: 30 cycles, HLA-DPB1: 32 cycles, HLA-G: 35 cycles, F5: 25 cycles). The detection of gene amount was performed on an automated laser-activated fluorescent DNA sequencer (ALF, GE Healthcare, Freiburg, Germany) according to the manufacturer's recommendations. Data were calculated using Fragment Manager software (GE Healthcare, Freiburg, Germany). Amplification of the HLA-DRB1 and -DQB1 genes was calculated from the ratio of peak areas using DYS205, which was added in a standard concentration and set at 100% as normalization. Each sample was amplified for its specific loci and alleles in 3 separate PCRs to control for PCR-dependent variations. Three independent gel runs of the identified loci were performed for every PCR to compensate for variations between different gels.

Comparative genomic hybridization (CGH)

For exclusion of more extensive chromosomal copy number alterations of chromosomes 6 and 1 in ovarian cancer tissue, comparative genomic hybridization (CGH) was carried out in 7 of 10 tumor samples that underwent the semiquantitative PCR-approach. In 3 cases, no additional DNA was available.

Metaphase spreads from a normal male subject were obtained from stimulated peripheral blood lymphocytes. CGH was carried out as described previously.²⁹ Image capture and processing for CGH analysis were performed using the Leica CW4000 CGH imaging system. The diagnostic threshold values used to score losses and gains were 0.75 (lower threshold) and 1.25 (upper threshold), respectively.³⁰ High level amplifications were defined as gains of chromosomal material, which led to either a very bright and distinct fluorescent band in the hybridization pattern of the tumor DNA and/or a deviation of the average ratio profile beyond the 2.0 threshold.

Immunohistochemistry

Expression and cellular localization of HLA-class II antigens (HLA-DR, -DQ, -DP) were evaluated immunohistochemically in 10 ovarian carcinomas. The monoclonal mouse anti-human MHC-class II antibody (clone CR3/43 (1:500 dilution), DAKO, Hamburg, Germany) was used, which is an IgG₁ that recognizes the β -chain of all products of the DP, DQ and DR subregions.³¹ Staining was performed on an automated staining system (TechMate 500, Dako, Hamburg, Germany) using the streptavidin-biotin complex method.

Immunoreactivity was analyzed *via* the standardized semiquantitative score of Remmele.³² Briefly, carcinomas were classified by the percentage of tumor cells with positive staining (no stained cells (0); <10% stained cells (1); 10–50% stained cells (2); 51–80% stained cells (3); >80% stained cells (4)). Compared to controls the intensity of expression was categorized as no (0), weak, (1) medium (2) and strong expression (3). The score was calculated by multiplication of the groups, resulting in a scale ranging from 0 to 12. A score of 0–1, which is equal to less than 10% stained nuclei with weak expression, was regarded as negative or neglectably low.

Tumor tissue sections expressing HLA-class II antigens were analyzed for lymphocytic infiltration and its association with HLA-class II positive cancer cells. Inflammatory reaction was considered to be present or absent evaluating peri- and intratumoral areas.

Statistical methods

Quantitative analysis included the following: comparison of amplification products for HLA-DRB1 and -DQB1 in normal and tumor tissue; determination of the extension of chromosomal changes by co-investigation of HLA-G and -DPB1. Gene amounts in tumor and normal tissue were normalized using the respective quantities of F5. For comparison we utilized an amplification ratio α , which was defined as follows:

$$\alpha = (N_T[X]/N_T[F5]) / (N_C[X]/N_C[F5]).$$

$N_T[X]$ denotes the quantity of the loci of interest (HLA-DRB1, -DQB1) in tumor and $N_C[X]$ in control tissue. $N_T[F5]$ and $N_C[F5]$ denote the quantity of the internal amplification control (F5) in corresponding tumor and control tissue. The distributions of tumor and control quantities were compared using the Student's *t*-test. Furthermore, comparisons between categorical variables were performed using the χ^2 -test looking for possible associations between gene amount and antigen expression or clinical parameters. Since only few cases were observed, the *p*-value was obtained by simulation procedure with the statistical software 'R' (version 2.1.0, The R Foundation for Statistical Computing, Vienna, Austria). Results with a *p*-value < 0.05 were considered to be significant.

HLA nomenclature

HLA-DRB1 and -DQB1 alleles were assigned according to the nomenclature provided by the 2004 report of the WHO Nomenclature Committee.³³ HLA-class II haplotypes were given consistent with published haplotypes in a Caucasian population.³⁴

Results

HLA-class II typing

DRB1-DQA1-DQB1 haplotypes were assigned to every patient (Table I). Haplotype frequencies are not representative, because we preferentially chose to investigate tissues from patients who carried the susceptibility haplotype DRB1*0301-DQA1*0501-DQB1*0201 (7/20). The second risk haplotype DRB1*1001-DQA1*0101-DQB1*0501 was present in one sample.

TABLE 1 – HLA-CLASS II HAPLOTYPES, HLA-CLASS II ANTIGEN EXPRESSION (DP, DQ, DR) ON TUMOR CELLS, PRESENCE OF TILs AND GENOMIC IMBALANCES IN 10 OVARIAN CARCINOMAS

ID	HLA-class II haplotype				HLA-class II expression				TILs		Genomic imbalances	
	DRB1	DQA1	DQB1	Staining intensity	Stained cells	Remmele score	Cellular location	Expression pattern	Presence/Absence	Association with HLA-class II positive tumor cells	Gain	Loss
1	0701 0301	0201 0501	0201 0201	1	1	1	C	H	Absence	-	1p32-p34, 2p11.2-p15, 3q26.1-q29, 7q32-q36, 8q23-q24.1, 12p11-p12, 12p13.3, 16p, 18q11.2-q21, 19q	1p36, 3p23-p26, 4p, 4q24-q35, 5q14-q31, 6q21, 8p21-p23, 9p, 11p15, 13q, 18q22-q23, Xp, Xq12-q24
2	0301 0301 0701	0501 0501 0201	0201 0201 0201	0	0	0	-	-	ND	ND	ND	ND
3	0301 0301	0501 0501	0201 0201	2	2	4	C	H	Presence	Association	ND	ND
4	0301 0401	0501 0301	0201 0301	3	4	12	D	H	Absence	-	5p, 8q22-q24, ² 20p13	1p36, 4q22-q35, 5q14-q23, 8p21-p23, 9p, 10q24-q26, 11q23-q25, 12q21, 13q22-q34, 16q, 18q21-q23
5	1501 0301 1401	0102 0501 0102	0602 0201 0602	1	1	1	C	H	Presence	No association	No Change	No Change
6	0801 0401	0401 0402	0402	2	3	6	CS	H	Absence	-	No Change	No Change
7	0401 1101	0301 0501	0302 0301	0	0	0	-	-	ND	ND	1q32-q44, 2, 6p21.3-p25, ¹ 7q, 8p, 8q23-q24, 12p, 17, 20	4q26-q34, 6q16-q27, 7p21-p22, 9p24, 10p15, 13q, 14q21-q24, 18q22-q23
8	1101 0301 1001	0501 0501 0101	0301 0201 0501	0	0	0	-	-	ND	ND	3q25-q29, 5p, 10p, 12, 18p	6q22-q27
9	0101	0101	0501	0	0	0	-	-	ND	ND	1p33-p36, 1q, ² 2, 3q, 6, ¹ 7q, 8p, 9q, 10p12-q26, 10p13-p15, 11p, 12, 16, 17q22-q25, 19, 20, 22q11.2-q12	4q21-q35, 5p, 5q11.2-q23, 9p, 11q, 18q23, X
10	0701 0401	0201 0301	0201 0302	1	1	1	C	H	Absence	-	ND	ND

C, cytoplasm; D, dot-like; CS, cell surface; H, heterogeneous; ND, not determined.

¹Genomic imbalances for 6p encoding the MHC system. ²Genomic imbalances for 1q encoding F5-³Hi high level amplification.

TABLE II - RATIOS OF ALTERED GENE AMOUNT (DRB1, DQB1, F5, HLA-G, DPB1) IN 10 OVARIAN CARCINOMAS GENERATED FROM COMPARISON OF QUANTITIES IN TUMOR AND NORMAL TISSUE WITH F5 SERVING FOR NORMALIZATION

	ID 1	ID 2	ID 3	ID 4	ID 5	ID 6	ID 7	ID 8	ID 9	ID 10
DRB1 0101	1.12 (0.7)	2.02 (0.0003)	0.21 (0.0002)	0.95 (0.8)	1.99 (0.008)	-	-	2.15 (0.03)	12.58 (0.00001)	-
DRB1 0301	-	-	-	1.09 (0.6)	-	-	3.11 (0.0002)	-	-	0.28 (0.0002)
DRB1 0401	1.38 (0.04)	-	0.86 (0.6)	-	-	1.03 (0.9)	-	-	-	0.13 (0.00004)
DRB1 0701	-	-	-	-	-	-	-	-	0.84 (0.4)	-
DRB1 0801	-	-	-	-	-	-	0.72 (0.04)	2.35 (0.0002)	-	-
DRB1 1001	-	-	-	-	-	6.19 (0.00007)	-	-	-	-
DRB1 1101	-	-	-	-	-	-	-	-	-	-
DRB1 1401	-	-	-	-	-	-	-	-	-	-
DRB1 1501	6.21 (0.00003)	3.76 (0.0002)	0.56 (0.008)	-	3.22 (0.001)	-	-	1.89 (0.0007)	-	0.21 (0.000007)
DQB1 0201	-	-	-	2.94 (0.0001)	1.98 (0.04)	-	1.03 (0.7)	1.65 (0.007)	-	-
DQB1 0301	-	-	-	2.72 (0.004)	-	-	5.39 (0.0002)	-	-	5.28 (0.000003)
DQB1 0302	-	-	-	-	-	0.99 (0.8)	-	-	-	-
DQB1 0402	-	-	-	-	-	-	-	-	3.28 (0.00002)	-
DQB1 0501	-	-	-	-	2.13 (0.003)	-	-	-	-	-
DQB1 0602	-	-	-	-	1.36 (0.06)	3.76 (0.00002)	1	1	1	1
F5 Exon 10	1	1	1	1	1	1	1	1	1	1
HLA-G Exon 2	2.05 (0.0004)	1.64 (0.2)	2.32 (0.008)	1.19 (0.4)	0.86 (0.3)	ND	ND	ND	ND	ND
DPB1 Exon 2	2.74 (0.00004)	1.36 (0.2)	1.09 (0.7)	1.27 (0.2)	-	ND	ND	ND	ND	ND

ND, not determined.

TABLE III - COPY NUMBER CHANGES OF HLA-CLASS II

Copy number changes	HLA-class II alleles DRB1 and DQB1 (n = 40)	HLA-class II haplotype DRB1-DQB1 (n = 20)	HLA-class II susceptibility haplotypes (n = 8)
Amplification	62.5% (n = 25)	50% (n = 10)	50% (n = 4)
Partial amplification ¹	-	20% (n = 4)	38% (n = 3)
Loss	17.5% (n = 7)	10% (n = 2)	12% (n = 1)
Partial loss ¹	-	10% (n = 2)	0% (n = 0)
Change in opposite direction ²	-	5% (n = 1)	0% (n = 0)
No significant change	20% (n = 8)	5% (n = 1)	0% (n = 0)

¹Partial amplification and partial loss implicate significant changes of gene amount in only one locus of the specific haplotype.²Concomitant loss and amplification in one haplotype is given as change in opposite direction.

Quantitative chromosomal changes of HLA-class II loci DRB1 and DQB1

Copy number changes of gene-specific DNA for the loci HLA-DRB1 and -DQB1 are given in Table II.

Significant alterations in the quantity of the specific PCR products were identified for 32 of the 40 alleles investigated (80%, Table III). In 25 alleles (62.5%) a significant gain, in 7 alleles (17.5%) a significant loss of gene amount was noted.

With regard to HLA-class II haplotypes, the following significant results were observed (Table III). In 10 haplotypes (50%) a gain of chromosome material with gene amplification for both DRB1 and DQB1 could be demonstrated. In 2 haplotypes (10%) a loss of chromosomal material was detected. In 4 haplotypes (20%) only one locus showed a gene amplification, whereas the other one remained unchanged. Similarly, in 2 haplotypes (10%) a loss of gene amount was identified in only one HLA-locus. One haplotype (5%) revealed countercurrent changes in the 2 HLA-loci defining the haplotype. In another haplotype (5%) no significant quantitative changes could be detected.

A closer look at the susceptibility haplotypes DRB1*0301-DQB1*0201 and DRB1*1001-DQB1*0501 revealed a significant amplification in 4 of 8 haplotypes (50%, Table III). In another 3 risk haplotypes (38%) we demonstrated amplification only for the DQB1 locus. In one case (12%) a loss of chromosome material was detected. In none of the risk haplotypes the gene amount in tumor tissue remained unchanged.

Quantitative chromosomal changes of HLA-G and -DPB1

No significant change in gene dosage in any of the 2 gene loci HLA-G and -DPB1 could be stated in 3 of the 5 patients investigated (60%, Table II). Significant copy number gain of both the HLA-G and -DPB1 genes was detected in one case, in which the haplotype DRB1*0701-DQB1*0201 was additionally amplified. In another case significant copy number gain was noted only for HLA-G whereas HLA-DPB1 remained unchanged and the HLA-class II loci DRB1 and DQB1 showed loss of chromosome material. In summary, long-distant alterations of chromosome 6p21.3 were excluded in the majority of the analyzed chromosomes.

Comparative genomic hybridization (CGH)

CGH analysis revealed chromosomal imbalances in 5 of 7 ovarian carcinomas (71%), with a range of 0-24 alterations per tumor (mean ± SEM: 12.1 ± 3.5, Table I). The most frequent recurrent gains/amplifications involved chromosomes 8, 12p and 20 (4/7, 57% each), 1, 2, 3q and 7q (3/7, 43% each), as well as 5p, 6, 10, 16, 17, 18 and 19 (2/7, 29% each). Recurrent losses mapped to chromosomes 4, 9p and 18q (4/7, 57% each), 5, 6q, 11 and 13q (3/7, 43% each), as well as 1p, 8p, 10 and X (2/7, 29% each). CGH

TABLE IV – ASSOCIATION BETWEEN HLA-CLASS II ANTIGEN EXPRESSION (DP, DQ, DR) ON TUMOR CELLS AND COPY NUMBER CHANGES OF HLA-CLASS II LOCI IN 10 OVARIAN CARCINOMAS

Expression of HLA-class II antigens	Copy number changes of HLA-class II alleles DRB1 and DQB1 (n = 40)		
	Amplification (n = 25)	LOH (n = 7)	No change (n = 8)
Without expression (score 0–1)	n = 21	n = 4	n = 3
With expression (score 2–12)	n = 4	n = 3	n = 5

p = 0.013

TABLE V – ASSOCIATION BETWEEN THE STAGE OF MALIGNANCY AND COPY NUMBER CHANGES OF HLA-CLASS II LOCI IN 10 OVARIAN CARCINOMAS

FIGO stage	Copy number changes of HLA-class II alleles DRB1 and DQB1 (n = 40)		
	Amplification (n = 25)	LOH (n = 7)	No change (n = 8)
≥FIGO III	n = 19	n = 0	n = 5
<FIGO III	n = 6	n = 7	n = 3

p = 0.0005

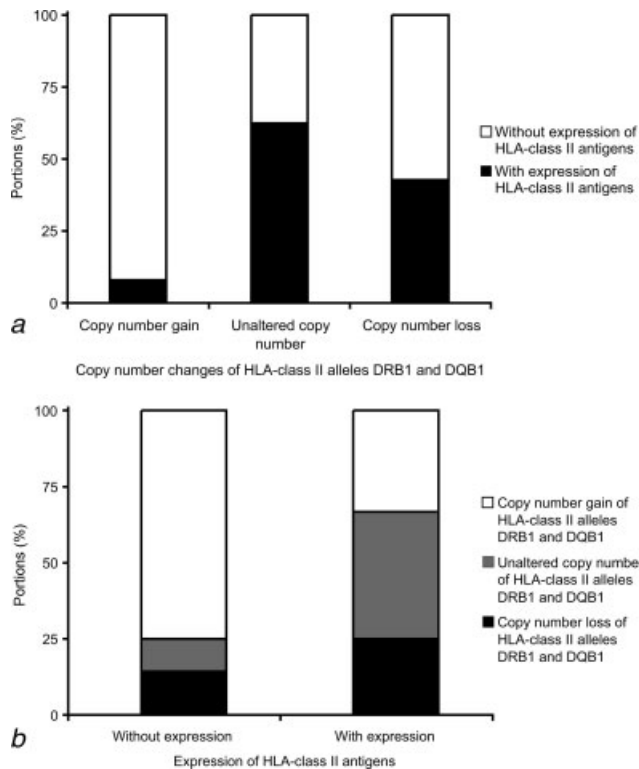


FIGURE 1 – Impact of gene copy number of HLA-class II alleles (DRB1, DQB1) on expression of HLA-class II antigens (DP, DQ, DR) in 10 ovarian carcinomas. a): The distribution of two classes of expression level of HLA-class II is given for different groups defined by their gene amount of HLA-class II alleles HLA-DRB1 and -DQB1. **(b):** The distribution of three classes of copy number change of HLA-class II alleles HLA-DRB1 and -DQB1 is given for different groups defined by their expression score.

revealed 3 tumors with high-level amplifications involving 3 genomic regions: 8q22–q24, 10p13–p15 and 12p13, respectively.

With regard to chromosome 6p encoding the MHC complex, genomic imbalances were excluded in the majority of analyzed ovarian cancers (71%). Chromosomal imbalances of 6p were detected in 2 of 7 tumors (29%) showing one gain of 6p21.3-p25 and one gain of the whole chromosome 6. Accordingly, in both instances HLA-class II haplotypes were amplified (DRB1*0401-DQB*0302, DRB1*0101-DQB*0501) while the flanking loci HLA-G and -DPB1 were not investigated.

With regard to F5, gain of 1q was observed in only one tumor that also showed gain of chromosome 6. Thus, any influence of

copy number changes at 1q23 on the quantitative analysis of HLA-class II loci could be excluded.

Immunohistochemistry

In 3 tumors (30%) HLA-class II antigen expression of cancer cells were found in varying degrees but consistently heterogeneous (Table I). TILs were identified in only one of these 3 tumors (Table I). In this case the inflammatory infiltrate was associated with HLA-class II positive tumor cells.

Expression level of HLA-class II was significantly associated with amplification status (*p* = 0.013, Table IV). At a closer look, reduced antigen expression was related to increased copy numbers of the corresponding HLA-genes and strong expression to unaltered gene amount (Fig. 1).

Clinicopathological parameters

The stage of malignancy according to the FIGO classification was significantly associated with the gene amount of HLA-class II loci DRB1 and DQB1 (*p* < 0.001, Table V). In particular, gene amplifications were more frequent in advanced tumor stages (FIGO ≥ III).

Discussion

To date, few data are available about molecular mechanisms responsible for altered HLA-class II phenotypes in tumors. In our study, copy number changes of HLA-DRB1 and -DQB1 and their linkage to HLA-class II antigen expression were investigated.

Our results showed chromosomal changes in the HLA-class II region to be frequent and gene or DNA amplifications to be more common than deletions. These data are in accordance with previous studies in serous adenocarcinomas in which gain of 6p was detected in 22% of tumors and loss in only 2%.³⁵ Moreover, gain of 6p was identified in serous and non-serous as well as high-grade and low-grade malignant ovarian carcinomas.³⁶ The minimal common region of chromosome 6p amplification was narrowed down to 6p21-22, which corresponds to the HLA-region.³⁷ Accordingly, copy number gain affects chromosome 6p in several tumor types and most of them map to the 6p21-p23 region.³⁸ Thus, it is possible that this region may harbor a yet unidentified oncogene.

Support comes from our results that the majority of susceptibility haplotypes DRB1*0301-DQB1*0201 and DRB1*1001-DQB1*0501, which were previously found to confer an increased risk for ovarian cancer,¹⁸ showed at least partial amplification of gene dosage. Accordingly, a recent analysis showed increased A2-B8-DRB1*03 haplotypes in ovarian cancer suggesting a more extensive HLA class I-II haplotype association with this disease.³⁹ Therefore, it could be theorized that the susceptibility haplotypes are in linkage disequilibrium with a yet unknown growth-regulating gene.

Oncogenes are assumed to represent an essential mechanism in cancer development. Indeed, we found amplifications to be more frequent in advanced-stage tumors. Correspondingly, a recent study showed that gain of 6p is associated with a poor prognosis and more common in stage IIIc than in stage IIIa and IIIb ovarian tumors.³⁵

Our data imply that the putative oncogene would be closely linked to the HKA-class II region since the evaluation of flanking gene-loci revealed that the majority of alterations observed were limited to the HLA-class II domain. Accordingly, genomic imbalances, which are the most frequent abnormality in cancer, were excluded for 6p in almost all analyzed ovarian cancers.

Several candidate oncogenes are localized on 6p. Especially, the vascular endothelial growth factor (VEGF) gene on 6p21.3 is assumed to play a role in the development of ovarian cancer.^{40,41} Additionally, the tumor necrosis factor- α (TNF- α) gene, which is located within the HLA-class III region of the MHC complex, is expected to promote cancer development.⁴²

Besides the identification of copy number changes within the HLA-region we aimed to elucidate their biological significance. We quantitatively assessed the degree of HLA-class II antigen expression on ovarian cancer cells. In our series, expression of HLA-class II molecules was observed in 30% of tumors. Different studies in ovarian cancer support our findings of *de novo* expression of HLA-class II antigens.⁴³⁻⁴⁵

It is well known, that the expression of HLA-class II on tumor cells might be due to cytokines produced by infiltrating inflammatory cells.^{10,11} In our sample TILs were found to be rare. Therefore, inducible effects from cytokines could be excluded in most cases. In accordance to these results, a recent study showed HLA-class II expression in thyroid cancer unrelated to lymphocyte infiltration suggesting an induction of antigen expression by regulatory mechanisms during carcinogenesis.¹²

Likewise, tumor-specific mechanisms may influence HLA-class II expression in our sample of ovarian carcinomas since correlation analysis between expression level and amplification status showed reduced levels of HLA-class II protein expression associated with increased copy numbers of the corresponding HLA-class II genes. Indeed, it has been shown, that copy number gain produced by an oncogene has an impact on HLA expression pattern. More specifically, the amplification of N-myc suppresses the

expression of HLA-class I antigens by reducing the binding of the p50 subunit of NF- κ B to the MHC-class I gene promoter.⁴⁶⁻⁴⁸

Further support for genetically determined immunological mechanisms of the tumor comes from the analysis of clinicopathological data, which reveals that gain of 6p21.3 is more common in advanced tumor stages and at the same time related to the absence of HLA-class II antigen expression. Thus, cancer cells may enhance aggressiveness by avoiding the upregulation of MHC antigens, consequently influencing tumor initiation and growth.

In summary, our investigation of the role of HLA-class II loci in cancerogenesis was initiated by the identification of HLA-class II susceptibility haplotypes for ovarian cancer. The analysis focused on copy number changes of 6p21.3 and antigen expression of HLA-class II molecules. Amplifications of the HLA-class II loci DRB1 and DQB1 were identified in the majority of ovarian carcinomas and they were limited to the HLA region. Accordingly, no gross chromosomal alterations were detected. The amplification status was inversely correlated to the expression level of the HLA-class II molecules. Whether this phenomenon reflects the presence of a near-by oncogene or the loss of cell differentiation in the course of tumor progression remains to be elucidated. Still, no significant differences of HLA-class II amplification or expression between carriers or non-carriers of the susceptibility haplotypes for ovarian cancer could be identified.

Further research in a larger patient sample will have to reconfirm the negative correlation between HLA-amplification and its expression level. Moreover, studies in borderline tumors and less advanced tumor stages will potentially clarify whether a causal relationship between HLA-expression status and aggressiveness of the corresponding tumor cells really exists.

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