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Association of Ovarian Tumor Epithelium Coexpressing HLA-DR and CA-125 Antigens with Tumor Infiltrating Cytotoxic T Lymphocytes

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Abstract

Understanding of the relationship between tumor infiltrating cytotoxic T lymphocytes (CD 8^+TILs) and tumor cells as well as tumor-associated antigens is important and may reflect the extent of immune response of the patient to the tumor, thus providing a useful clue relevant to the prognosis. The purpose of this study was to determine the relationship between the expression of HLA-DR and CA-125 antigens and the presence of CD 8^+TILs with regard to the established clinicopathological factors of ovarian carcinomas using immunohistochemical methods. Thirty-one ovarian carcinomas consisting of 20 serous, 7 mucinous, and 4 clear cell types were examined. Of these, 18 (58%) and 22 (71%) were positive for HLA-DR and CA-125 antigens respectively, and the overall mean number of the CD 8^+TILs was 7.2 ± 2.9. A significant association was observed between the mean number of CD 8^+TILs and tumor grade ($P = 0.01$), disease stage ($P = 0.003$), and patient outcome ($P = 0.01$). The mean number of CD 8^+TILs in HLA-DR positive (8.6 ± 2.2) or CA-125 positive (8.4 ± 2.1) tumors was significantly higher than that in HLA-DR negative (5.2 ± 2.5; $P = 0.0003$) or CA-125 negative (4.2 ± 2.2; $P = 0.00002$) tumors. These signifcant levels were further enhanced by one order of magnitude when the mean number of CD 8^+TILs in tumors postive for both HLA-DR and CA-125 antigens (9.1 ± 1.7) was compared to that in HLA-DR negative or CA-125 negative tumors. The frequency of cancer-related deaths in HLA-DR and CA-125 positive tumors was significantly lower than in the negative tumors ($P = 0.01$). These data suggest that concurrent expression of HLA-DR and CA-125 antigens may augment the immune response of the patient to the tumor, thus providing a potential clue relevant to the prognosis.
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Key words: ovarian tumor, antigens, HLA-DR, CA-125, tumor infiltrating cytotoxic T lymphocytes, TILs, CD 8

Introduction

Ovarian carcinoma holds the highest mortality rate among gynecological malignancies. About two-thirds of patients suffer from an advanced disease at the time of diagnosis. Currently, established prognostic indicators of ovarian carcinoma include: disease stage, histologic grade, residual tumor volume, and response to chemotherapy. Identification of additional prognostic factors that in association with established clinicopathologic parameters predict which patients are more likely to develop tumor progression is highly important for

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an effective therapy.

The major histocompatibility complex (MHC) consists of a group of genes located on chromosome 6 in humans which encode several immunoregulatory molecules including HLA antigens[1]. HLA-DR molecules regulate the ability of cytotoxic T lymphocytes (CD 8$^+$TILs) to recognize various antigens, whether viral or tumoral. The expression of HLA-DR on tumor cells has been shown to be associated with a favorable prognosis[2,3]. On the other hand, CA-125 antigen is an ovarian carcinoma-associated antigen which has been widely used as a diagnostic and prognostic marker[4,5]. The expression of HLA-DR and CA-125 antigens in ovarian carcinoma cell lines has been found to be regulated by interferons[6]. It has been shown that a combination of interferon-gamma and cisplatin results in a synergistic amplification of antiproliferative activity[7]. Subsequently, the expression of HLA-DR and CA-125 antigens has been shown to be markedly augmented by interferon-gamma[7]. Additionally, in a preliminary study on frozen tissue sections from ten ovarian carcinomas (unpublished data), we noted that the majority of cases showed simultaneous expression of HLA-DR and CA-125 antigens in association with the presence of CD 8$^+$ TILs. These findings prompted us to hypothesize that a concurrent expression of HLA-DR and CA-125 antigens in ovarian carcinomas may confer an enhanced immune response. The purpose of this study was to determine the relationship between the expression of HLA-DR and CA-125 antigens and the presence of CD 8$^+$TILs in ovarian carcinomas with regard to the established clinicopathological factors of the disease.

Materials and Methods

Tissues

Formalin-fixed, paraffin-embedded tissue sections from 31 ovarian carcinomas (20 serous, 7 mucinous, and 4 clear cell type) were studied. The use of the paraffin sections had been approved by the Institutional Review Board. Serial 4-μm sections from the tumors were prepared. A section from each tumor was stained with hematoxylin and eosin to verify the tissue and diagnosis. Histological types and grades were determined using the World Health Organizatin Criteria[8], and the stage of tumors was assessed according to the International Federation of Gynecology and Obstetrics staging system[9]. All patients in this study received postoperative chemotherapy for a high-risk early stage (stage I, grade 3; stage IC; any stage II) or advanced diseases (stages III and IV). The histopathologic types and grades and clinical stages of the patients are summarized in **Table 1**. There were 20 serous, 7 mucinous, and 4 clear cell type tumors. The distribution of tumors according to the grade (excluding the 4 cases with clear cell carcinomas) and stage were: 8 grade 1,9 grade 2, and 10 grade 3; and 6 stage I, 11 stage II, 9 stage III, and 5 stage IV tumors. Of the 31 ovarian carcinoma patients, 18 were alive with no evidence of the disease and 13 had died of the disease at the time of analysis. The median follow-up for all patients was 28 months (range, 6 to 82).

Immunohistochemistry

Immunostaining for HLA-DR, CA-125 and CD 8 antigens was performed using an avidin-biotin-peroxidase complex method[10]. Endogenous peroxidase was blocked in deparaffinized tissue sections by 3% hydrogen peroxide in methanol for 20 minutes. After washing with phosphate buffer saline (PBS), pH 7.4, the tissue nonspecific binding sites were blocked by 10% normal goat serum for 20 minutes. The sections were then incubated with monoclonal anti-HLA-DR (1: 100), CA-125 (1: 100), or CD 8 (1: 50) antibody (Dako Japan) overnight at 4℃. After washes with PBS, biotinylated goat anti-mouse IgG (1: 600, Dako) was applied followed by washing in PBS and incubation with an avidin-biotin-peroxidase complex reagent (1: 800, Dako). Diaminobenzidine was used as chromogen. The sections were counterstained briefly with Mayer's hematoxylin, dehydrated, and mounted. Negative controls included the use of a non-immune rabbit immunoglobulin as the primary antibody. As positive controls, we used sections from a serous ovarian carcinoma known to be positive for CA-125 antigen and a breast adenocarcinoma tissue known to be positive for HLA-DR and tumor infiltrating CD 8$^+$T lymphocytes.

Interpretation of Immunostaining

Evaluation of immunostained sections was done without knowledge of the clinical status of the patients. In each case, the mean percentage of immunostained epithelial tumor cell area for HLA-

Table 1 Association of HLA-DR and CA-125 antigen expressions and mean number of $CD8^+$ TILs with the clinicopathologic factors

Histology	No.	$HLA\text{-}DR^+$	$CA\text{-}125^+$	$CD8^+$ TILs (Mean ± SD)
All cases	31	18 (58%)	22 (71%)	7.2 ± 2.9
Cell type				
Serous	20	13 (65%)	16 (80%)	7.7 ± 3.0
Mucinous	7	4 (57%)	4 (57%)	6.3 ± 1.8
Clear cell	4	1 (25%)	2 (50%)	5.9 ± 3.1
Grade*				
1	8	3 (38%)	5 (63%)	5.4 ± 2.6
2	9	7 (78%)	7 (87%)	7.8 ± 2.6
3	10	7 (70%)	8 (80%)	8.6 ± 2.6
Stage†				
Ⅰ	6	1 (17%)	3 (50%)	5.0 ± 2.9
Ⅱ	11	5 (45%)	7 (64%)	6.4 ± 2.4
Ⅲ	9	7 (78%)	7 (78%)	7.8 ± 2.6
Ⅳ	5	5 (100%)	5 (100%)	10.3 ± 0.9
Patient outcome‡				
Alive	18	14 (78%)	17 (94%)	8.2 ± 2.3
Died	13	4 (31%)	5 (38%)	5.8 ± 3.0

*: Excluding 4 cases with clear cell carcinomas; grade 1 vs. 3: $CD8^+$ TILs, $P = 0.01$.
†: Stage Ⅰ vs. Ⅳ : $CD8^+$ TILs, $P = 0.003$. ‡: Alive vs. died: $HLA\text{-}DR^+$, $P = 0.01$; $CA\text{-}125^+$, $P = 0.001$; $CD8^+$ TILs, $P = 0.01$.

DR and CA-125 antigens, and the mean number of CD 8^+TILs within the tumor in comparable areas in serial sections for five microscopic fields at 400 x magnification were determined. Tissue sections with more than 20% of the tumor cells showing positive staining for HLA-DR or CA-125 were regarded as positive.

Statistical analysis

The frequencies of HLA-DR and CA 125 antigen positivity in relation to various clinicopathologic factors were compared using the Fisher's exact test. The mean numbers of CD 8^+TILs were compared using the Student's *t* test for unpaired data. All statistical analyses were performed using the SPSS statistical software system (SPSS Inc., Chicago, IL). A probability (P) value of less than 0.05 was considered as significant.

Results

Fig. 1 depicts the representative immunohistochemical expressions for HLA-DR and CA-125 antigens as well as the CD 8^+TILs in a serous ovarian carcinoma. Immunostaining for HLA-DR antigen was confined to the intraluminal tumor cell surfaces and some stromal cells. The CA-125 antigen was diffusely localized on the tumor cell membranes. The CD 8^+TILs were distributed within the tumor areas and especially around the tumor cell nests in the stroma. **Table 1** summarizes the results in relation to the clinicopathological factors of ovarian carcinomas. Of the 31 ovarian carcinomas, 18 (58%) and 22 (71%) were postive for HLA-DR and CA-125 antigens respectively, and the overall mean number of the CD 8^+TILs was 7.2 ± 2.9. A significant correlation was found between the expression of HLA-DR antigen ($P = 0.01$) or CA-125 antigen ($P = 0.001$) and patient outcome, but not histologic types, tumor grade, of disease stages. Also, a significant difference in the mean number of CD 8^+TILs was observed between the patients who were alive and those who died ($P = 0.01$), and when grade 1 and 3 tumors ($P = 0.01$) or stage I and IV tumors ($P = 0.003$) were compared. The mean number of CD 8^+ TILs in HLA-DR positive (8.6 ± 2.2, n = 18) or CA-125 positive (8.4 ± 2.1, n = 22) tumors was significantly higher than that in HLA-DR negative (5.2 ± 2.5, n = 13; $P = 0.0003$) or CA-125 negative (4.2 ± 2.2, n = 9; $P = 0.00002$) tumors (**Table 2**). The mean number of $CD8^+$TILs in tumors concurrently postive for HLA-DR and CA-125 antigens (9.1 ± 1.7, n = 16)

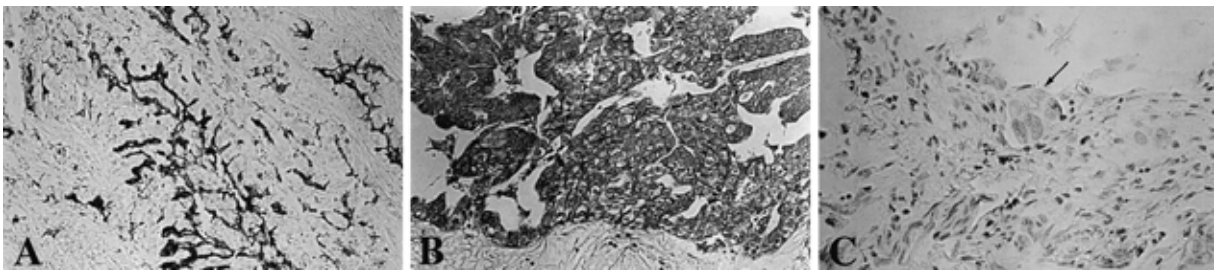

Fig. 1 Representative immunohistochemical demonstration of HLA-DR and CA-125 antigens as well as the infiltrating CD 8$^+$T lymphocytes in a serous ovarian carcinoma. (A) HLA-DR positivity was confined to the intraluminal surfaces of the tumor cells and some stromal and infiltrative cells. (B) CA-125 antigen was diffusely localized on the epithelial tumor cell surfaces. (C) Infiltrationg CD 8$^+$T lymphocytes were distributed within the tumor areas and often around the tumor cell nests (arrow) in the stroma. A, B: ×200; C: ×400.

Table 2 Association of HLA-DR and CA-125 antigen expressions with the mean number of CD8$^+$ TILs

Tumor	No. Cases	CD8$^+$ TILs (Mean ± SD)	*P* value
HLA-DR$^+$	18 (58%)	8.6 ± 2.2	0.0003
HLA-DR$^-$	13 (42%)	5.2 ± 2.5	
CA-125$^+$	22 (71%)	8.4 ± 2.1	0.00002
CA-125$^-$	9 (29%)	4.2 ± 2.2	
HLA-DR$^+$, CA-125$^+$	16 (52%)	9.1 ± 1.7	0.00003
HLA-DR$^-$	13 (42%)	5.2 ± 2.5	
HLA-DR$^+$, CA-125$^+$	16 (52%)	9.1 ± 1.7	0.000002
CA-125$^-$	9 (29%)	4.2 ± 2.2	

showed a further increase in significance level when compared to that in HLA-DR negative (5.2 ± 2.5, n = 13; $P = 0.00003$) or CA-125 negative (4.2 ± 2.2, n = 9; $P = 0.000002$) tumors.

Discussion

In the present study, we observed a significant association between the positivity for HLA-DR or CA-125 antigen and the mean number of CD 8$^+$TILs. This association was further strengthened when a combined phenotype of HLA-DR and CA-125 antigen positiviy was considered. This finding suggested a supplementary role for CA-125 antigen expression in attracting CD 8$^+$TILs to the site of the tumor or augmenting the local immune cell response. Further more, the expression of HLA-DR and CA-125 antigens revealed a significant association with patient outcome, and the mean number of CD 8$^+$ TILs showed a significant correlation with patient outcome. With regard to the tumor grade and stage, we found a significant association with CD 8$^+$TILs if grade 1 vs. 3 or stage I vs. IV only was compared. However, this association was voided if other grouping combinations were considered.

Ottenhoff et al.[11] showed that HLA-DR molecules are the main restriction determinants for antigen presentation. Subsequently, the prognostic role of HLA-DR antigen expression alone or in relation to the presence of CD 8$^+$TILs was explored in tumors from several organs[12–16]. In a study on HLA-DR expression in 100 patients with large bowel carcinomas, a strong expression was associated with the best survival[12]. In 68 laryngeal carcinomas, HLA-DR positive tumors showed a good prognosis[13]. In a study on 70 consecutive patients with esophageal carcinomas, the presence of CD 8$^+$TILs was associated with a good prognosis in both squamous and adenocarcinomas[14]. Our own study revealed that the frequency of cancer-related deaths and the mean number of CD 8$^+$TILs in HLA-DR positive tumors were significantly lower than in the negative tumors. These findings suggest that the expression of HLA-DR antigen by tumors or the abundance of CD 8$^+$

TILs may Indicate a favorable prognosis.

In ovarian carcinomas, it was shown that TILs including CD 8$^+$lymphocytes could recognize autologous tumors in an MHC class II-restricted (HLA-DR) fashion[15]. In 20 ovarian carcinomas, the proportions of HLA-DR positive tumor cells were found to be correlated positively only with the number of CD 8$^+$TILs[16]. Furthermore, studies on ovarian carcinoma cell lines have shown that gamma-interferon mediates the presentation of CA-125 antigen on the cell surface[6]. Interferons are known to modulate several cellular functions by the induction of different proteins. The HLA-DR expression was also found to be regulated by interferons, but in a different way. On the other hand, a combination of interferon-gamma and cisplatin resulted in a synergistic amplification of antiproliferative activity in ovarian carcinoma cells, and the expression of HLA-DR and CA-125 antigens was markedly augmented by induction with gamma-interferon[7]. We also found that the mean number of CD 8$^+$TILs (representing local immune cell response) was significantly higher in HLA-DR positive than in HLA-DR negative ovarian carcinomas. In addition, we observed a similar trend with regard to the expression of CA-125 antigen.

Taken together, the above results suggest that concurrent expression of HLA-DR and CA-125 antigens may augment the immune response of the patient to the tumor, thus providing a potential clue relevant to the prognosis.

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