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Altered expression of cellular membrane molecules of HLA-DR, HLA-G and CD99 in cervical intraepithelial neoplasias and invasive squamous cell carcinoma

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Abstract

Objective: The aim of this study was to investigate the role of HLA-DR, HLA-G and CD99 during cervical carcinogenesis and to examine the prognostic significance of these protein expressions in invasive squamous cell carcinoma (SCC).
Methods: Using specific antibodies for HLA-DR, HLA-G and CD99, we examined protein expressions in 19 normal cervix, 15 mild dysplasia (CIN I), 22 moderate dysplasia (CIN II), 23 severe dysplasia (CIN III), and 34 invasive squamous cell carcinoma by immunohistochemistry. And we detected the expression of Ki67 in the same specimens.
Results: None of normal cervix and CINs except three cases of CIN III expressed HLA-DR. HLA-DR expression increased progressively with the grade of the tumor, and significant differences could be observed between grade 1 and grade 2 ($P<0.01$) and between grade 1 and grade 3 ($P<0.05$). In all normal epithelial control samples, HLA-G expression was seen in ectocervical squamous and endocervical columnar epithelium and the staining was strong and uniform. Only a small proportion of CINs and SCCs showed reduced expression of HLA-G. Compared with the results in the control samples, CINs and SCCs showed significantly reduced expression of HLA-G ($P<0.001$). SCCs showed significantly increased expression of CD99 when compared with normal cervix and CINs ($P<0.05$). Ki67 was expressed in all specimens. Significant differences were observed between CINs and normal cervix ($P<0.001$) and SCCs and controls ($P<0.001$), but no significant differences could be observed between SCCs and CINs. None of the expressions of these proteins was associated with any of clinicopathological parameters.
Conclusions: These results indicate that increased expression of HLA-DR and CD99 may be related to the evolution of cervical cancer. All protein expressions were not associated with clinicopathological parameters.

Keywords: HLA-G; HLA-DR; CD99; Ki67; Cervical neoplasms

Introduction

Cervical cancer is the second most common cancer among women, being responsible for 400,000 deaths worldwide every year. The vast majority of carcinomas originate within the transformation zone of the cervix uteri, where proliferating cells are exposed at the squamocolumnar junction. Cervical intraepithelial neoplasia (CIN) is characterized by abnormal cellular proliferation, abnormal maturation as manifested by loss of polarity, cellular disorganization, and nuclear atypia. The CIN grading classifies these lesions into grades I, II, or III, corresponding to mild, moderate, or severe dysplasia/carcinoma in situ (Wright et al., 1994). In many cases, mildly and moderately dysplastic areas (CIN I and CIN II) regress spontaneously (Spriggs and Boddington, 1980), while CIN III is regarded as being identical to carcinoma in situ (CIS), and about 50% of which may develop to an invasive cervical carcinoma (Fidler et al., 1968). There is now compelling evidence for the causative role of human papilloma infection (HPV) in the disease (Zur-Hausen, 1999). HPV DNA has been

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demonstrated in more than 99.7% of tumor biopsy specimens (Munoz, 1997). It appears that infection with HPV predisposes to cervical cancer in a multistage tumorigenic process. The role of the immune response in the pathogenesis of HPV-associated neoplasia is still poorly studied, although its significance has suggested by indirect and in vivo evidences: increased incidence of cervical neoplasia observed among immunosuppressed patients (Benton et al., 1992); localized immune dysfunction characterized by reduction of CD4 T-helper lymphocytes in lesions of HPV-associated carcinomas, and regression of HPV-induced lesions accompanied by tissue infiltration with macrophages, cytotoxic T cells, and natural killer cells (Viac et al., 1993).

The mechanisms of inhibited immune response of the host against tumor involve disorders of number and function of immune cells, and altered expression of cellular membrane molecules of tumor cells, which prevents recognition and elimination from host immune cells. The molecules affecting recognition by immune cells include membrane histocompatibility-related leukocyte antigens (HLA) Class I and II. HLA molecules are known to play a significant role in stimulating and regulating the immune response. Recent studies reveal that HLA-DR, HLA-G, and CD99 are also important in immune response.

The aims of the present study were to determine the frequency and tissue distribution patterns of the HLA-DR, HLA-G, CD99 in normal cervix, CINs and invasive squamous cervical cancer, to evaluate the expression of HLA-DR, HLA-G, CD99 as markers for cervical carcinogenesis, and to examine the prognostic significance of these protein expression in invasive squamous cell carcinoma.

Materials and methods

Study group

The study subjects enrolled between November 2002 and April 2004 from Center of Clinical Sample Resource Library, Women's Hospital, School of Medicine, Zhejiang University. None of patients received chemotherapy or radiotherapy before operation. The study subjects included 94 patients with cervical lesions, 15 mild dysplasia (CIN I) with the age of 26–64 years, 22 moderate dysplasia (CIN II) with the age of 30–52 years, 23 severe dysplasia (CIN III) with the age of 30–71 years, 34 invasive squamous cell carcinoma (SCC) with the age of 26–71years. Of the 34 SCCs, 21 were Stage I, 13 were Stage II. Histopathologically, 10 were grade 1, 14 were grade 2, 10 were grade 3. All of the 34 SCCs were treated by radical hysterectomy and bilateral pelvic lymphadenectomy at Women's Hospital, School of Medicine, Zhejiang University. All patients were preoperatively examined and tumor diameter was assessed by inspection and palpation. No preoperative screening for lymph node metastasis was done. A range of 18–30 lymph nodes were removed and no patient was found to have lymph nodes metastasis. Only one patient was found to have parametrial invasion. Patients were followed until June 2005 and none was lost to follow-up. No patient had a relapse during this short period of follow-up. The samples were collected in 10% buffered formalin and embedded in paraffin within 24 h for long-term preservation.

In order to obtain cytologically normal control subjects, 19 normal uterine cervical epithelium were obtained from age-matched (36–68) patients undergoing hysterectomy for various nonmalignant diseases. All of the original haematoxylin and eosin-stained slices were blindly reviewed by two experienced histopathologists.

Immunohistochemical staining for HLA-DR, HLA-G, CD99 and Ki67

HLD-DR (Mob 069, CR3/43) and CD99 (M0b 262, HO36-1.1) monoclonal mouse antibody were purchased from DBS Biotechnology (Pleasanton, CA), HLA-G monoclonal mouse antibody (MEM-G/1) from BioVendor Laboratory Medicine, Inc. (Brno, Czech Repubic), and Ki67 (MIB-1) from Gene Co. (Dako, Dakopatts, Glostrup, Denmark). The staining procedure followed strictly the guideline of EnVision Plus-HRP kit (Dako, Dakopatts, Glostrup, Denmark). Simplified as follows, adjacent sections of 4 μm thickness were cut and placed on a poly-L-lysine-coated glass slides, air-dried overnight at 60 °C. The sections were dewaxed in xylene and rehydrated in graded ethanol (100%, 95%, 85%, and 75%) for 5 min each, then rinsed in water. Slides were then incubated for 30 min in 3% hydrogen peroxide in methanol to quench endogenous peroxidase activity and were then pressure-cooked for 2 min for antigen retrieval in 0.01 M citric acid buffer. The sections were reacted with the primary antibody (dilution: 1:150 for HLA-DR; 1:70 for HLA-G; 1:25 for CD99; 1:150 for Ki67) for 2 h and then incubated with a commercially available EnVision Plus-HRP kit for 30 min. The color was developed with 3-3′-diamnobenzidine (DAB), and the sections were eventually counterstained with Harris' haematoxylin. All series included positive controls. Appropriate validation (negative controls) was performed by replacing the primary antibody with PBS alone.

Evaluation of results of IHC staining

The scoring method of Sinicrope et al. (1995) was applied to evaluate the IHC staining intensity and the proportion of stained epithelial cells. Membrane staining and nuclear staining were considered independently. For evaluation of HLA-DR, HLA-G and CD99, the staining intensity was subclassified as 1, weak; 2, moderate; and 3, strong. The numbers of positive cells were expressed as the percentage of the total number of epithelial cells and assigned to one of five categories: 0, <5%; 1, 5–25%; 2, 26–50%; 3, 51–75%; 4, >75%. The percentage of positivity of tumor cells and the staining intensity were multiplied to produce an immunoreactive score (IS) for each specimen. To determine the Ki67 labeling index of all samples, immunohistochemical stained slides were randomly moved and positioned to encompass the full thickness of the epithelium with the highest labeling. The number of positively labeled