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Up-regulation of Human Leukocyte Antigen G Expression in Primary Cutaneous Malignant Melanoma Associated with Host-vs-tumor Immune Response

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Summary: Human leukocyte antigen G (HLA-G) is one of the molecules implicated in immunotolerance. To investigate the role of HLA-G in primary cutaneous malignant melanoma (CMM), a series of 47 skin melanocytic lesions were immunohistochemically evaluated. The correlation between HLA-G expression and CMM clinicohistopahtological data and Bcl-2 expression was also analyzed. HLA-G expression was detected in a variety of cell types. No significant difference in HLA-G expression was observed between malignant and non-malignant melanocytic lesions. HLA-G expression was significantly correlated with the inflammatory infiltration and Bcl-2 expression, whereas no significant correlation with ulceration, tumor thickness, clinical stage, histopathological subtypes were observed. HLA-G expression may be the result of host immune reaction in tumor microenvironment rather than a malignant feature of CMM.
Key words: cutaneous malignant melanoma; human leukocyte antigen G

Human leukocyte antigen G (HLA-G) is one of the class Ⅰ molecules of non-classic major histocompatibility complex characterized by tissue-restrictive distribution and limited polymorphism. The expression of HLA-G was noted in extra-villous trophoblasts, fetal capillary endothelia, adult cornea and thymic epithelia. It is also detected in tumors such as melanoma, breast, renal and lung carcinomas, glioma, and cutaneous lymphomas[1]. HLA-G is believed to be able to maintain immunologically privileged sites where the tissue is protected from the autoimmune attack. It has been postulated that up-regulation of HLA-G, like Bcl-2, confers tumors a selective advantage[2,3]. In this study, we immunohistochemically examined a series of specimens of skin melanocytic lesions to determine the level of HLA-G expression and to find its associations with various prognostic variables in primary cutaneous melanoma (CMM).

1 MATERIALS AND METHODS

1.1 Tissue Samples

The specimens of skin melanocytic lesions were surgically removed, formalin-fixed, paraffin-embedded and immunohistochemically examined. The patients involved came from the Union Hospital and Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, and from the First College of Clinical Medicine of Three Georges University, China, from 1998 to 2006. The diagnoses of all the specimens were histopathologically and clinically confirmed. Of the 47 melanocytic lesions, 35 were of primary cutaneous malignant melanoma (CMM) and 12 nevocellular naevi. Clinicopathological data, including site, clinical stage, Clark level, Breslow tumor thickness (classified as 1 when ≤1.00 mm and 2 when >1.00 mm), ulceration, growth pattern (radial growth phase and vertical growth phase), inflammatory infiltration (scored as 0: absent; 1: non-brisk; 2: brisk), lymph node metastasis, were available for all of the patients. Of the 12 samples of nevocellular naevi, 6 were of common type and 6 had inflammatory infiltration (3 halo nevi and 3 infected nevocellular naevi). A control group consisting of 8 skin biopsies of normal adult was also examined.

1.2 Antibodies

The antibodies used included anti-HLA-G mAb MEM-G/1 (Exbio, Czech Republic) and anti-Bcl-2 mAb (Boster Bio-Tech Co., Ltd, China)

1.3 Immunohistochemistry

Immunohistochemical staining was routinely performed by using an avidinbiotin peroxidase complex (ABC) method as described previously[3]. 3-amino-9-ethylcarbazole (AEC, Boster Bio-Tech Ltd., USA) solution (0.2 mg/mL AEC in 0.005 mol/L acetate buffer containing 0.03% perhydrol, pH 5.0) was used as chromogene. Slides were mounted with Aquamount. Chorionic carcinoma specimen was used as positive control.

1.4 Evaluation of HLA-G and Bcl-2 Expression

Expression of HLA-G was taken as positive if the membrane and cytoplasm of tumor cells were stained bright red. The expression of HLA-G and Bcl-2 were

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semi-quantitatively determined in terms of proportion of positive cells and divided into 3 groups: no expression (–), 1%–25% tumor cells expression (weak expression, +), and more than 25% expression (strong expression,++).

1.5 Statistical Analysis

All statistical analyses were conducted by using SPSS12.0 software package. The relations of clinicopathological variables to HLA-G were analyzed by employing Fisher's exact test. Spearman rank correlation test was used for the analysis of non-parametric variables. Differences at $P<0.05$ were regarded as statistically significant.

2 RESULTS

2.1 Expression of HLA-G Antigen

The staining of HLA-G was observed at the membrane and cytoplasm of melanoma cells, non-malignant melanocytic cells, inflammatory infiltrating cells, endothelial cells and eccrine gland epithelial cells (fig. 1–3). In melanoma, the positive staining was scattered and heterogeneous in tumor nests. Of 47 skin melanocytic lesions, HLA-G was detected in 12/35 (34.2%) of primary CMM and 2/6 (33.3%) of inflammatory naevi, and but not in common naevi. No significant difference was observed between CMM and naevi.

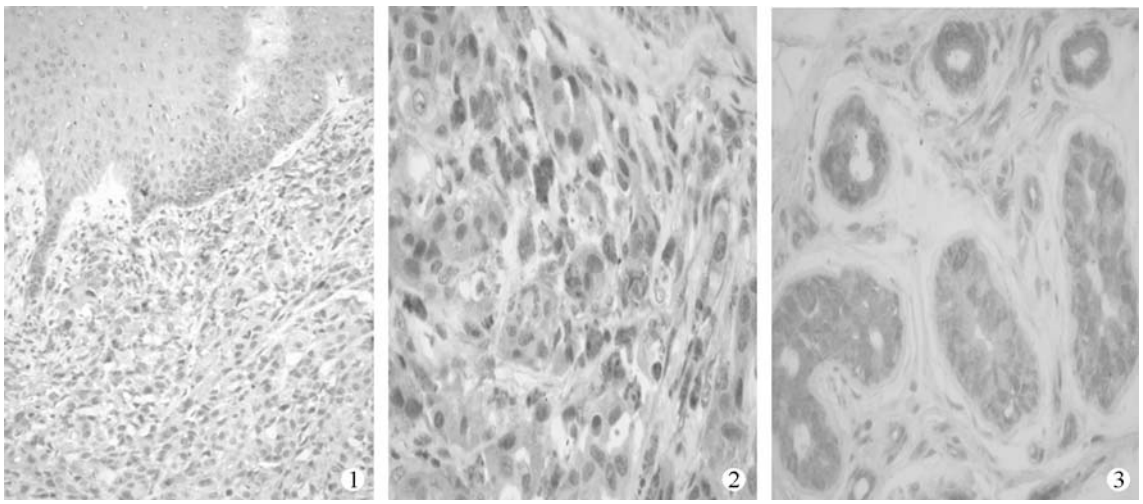

Fig. 1 HLA-G is expressed in a heterogeneous pattern in melanoma cells and infiltrating mononuclear cells (SP×200)
Fig. 2 HLA-G is expressed in melanoma cells (SP×400)
Fig. 3 HLA-G is expressed in adjacent eccrine gland (SP×400)

2.2 Expression of Bcl-2 Protein

Bcl-2 staining was observed at the cellular membrane and cellular nucleus. Of 35 specimens of primary cutaneous melanomas, 18 (51.4%) showed weak Bcl-2 expression, 6 (17.2%) strong expression and 11 (31.4%) had no expression.

2.3 Association between HLA-G Expression in CMM and Clinicohistopathological Variables

HLA-G expression in CMM was significantly correlated with the inflammatory infiltration (absent vs non-brisk and brisk combined, $P=0.032$). There was no significant correlation between HLA-G expression and clinical stage (stage Ⅰ–Ⅱ vs stage Ⅲ–Ⅳ), Clark level, Breslow tumor thickness, ulceration, growth pattern.

2.4 Correlation of HLA-G and Bcl-2 Expression in Primary Cutaneous Melanoma

Within CMM, a significant correlation was observed between expression of HLA-G and that of Bcl-2 ($r=0.440$, $P=0.008$).

3 DISCUSSION

Our study showed that HLA-G was expressed in 34.2% of CMM, which was consistent with previous observations[3]. HLA-G staining was noted in a variety of cell types, including melanoma cells, non-malignant melanocytic cells, inflammatory infiltrating cells, endothelial cells located in same tumor microenvironment, and eccrine gland adjacent to tumor. No significant difference in HLA-G expression was observed between malignant and non-malignant melanocytic lesions as previous study revealed[3]. Tumor microenvironment includes structural compartments, such as neoplastic, non-neoplastic cells and extracellular matrix, as well as soluble compartments, e.g., cytokines, chemokines, and polypeptide growth factors[4]. HLA-G expression could be triggered in response to the change of tumor microenvironment.

Tumor-infiltrating inflammatory cells are critical in modulating tumor microenvironments and contribute to tumor progression. The presence of HLA-G was found in some tumors, including CMM as well as some autoimmune and inflammatory diseases, but its was seldom found in normal tissues[5]. In this study, this molecule was also observed to be expressed in inflammatory naevi, and significant correlation existed between HLA-G expression and presence of the inflammatory infiltration. Therefore, HLA-G expression in CMM may be a common feature of host-vs-tumor response rather than an

indicator of malignancy.

Bcl-2 belongs to a family of apoptosis-blocking proteins. It has been postulated that up-regulation of Bcl-2 expression confers tumors a selective advantage, but studies failed to demonstrate such an association in CMM[6]. Our study demonstrated that Bcl-2 expression was significantly correlated with that of HLA-G, suggesting that HLA-G expression is probably the consequence of host-vs-tumor immunity within the tumor microenvironment at certain tumor stages, and other immunophenotypes of CMM, such as Bcl-2 expression, may also experience change as well.

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