ELSEVIER

Application of Human Leukocyte Antigen–G Expression in the Diagnosis of Human Cancer

Ie-Ming Shih

ABSTRACT: It has been well known that human leukocyte antigen (HLA)–G molecules are present in a variety of human neoplastic diseases, and the molecule may contribute to the escape of tumor cells from immune surveillance. Besides the studies that aim at elucidating the roles of HLA-G in immune regulation, the researches that focus on potential applications of HLA-G expression in cancer diagnosis represent another perspective in HLA-G research. This review summarizes those recent translational studies of HLA-G expression in the diagnosis of human cancer. Specifically, the promises and challenges for applying HLA-G expression to detect cancer in body fluids, to diagnose different types of human cancer and to predict clinical outcome in cancer patients will be briefly reviewed. *Human Immunology 68, 272–276 (2007).* © American Society for Histocompatibility and Immunogenetics, 2007. Published by Elsevier Inc.

KEYWORDS: human cancer; diagnosis; detection; prognosis

ABBREVIATIONS

ELISA	enzyme-linked immunoassay
HLA-G	human leukocyte antigen–G
sHLA-G	secreted HLA-G

INTRODUCTION

Recent studies have provided ample evidence that the human leukocyte antigen (HLA)–G molecule participates in immune regulation, especially in immune tolerance in physiologic process such as pregnancy. Shortly after the identification of HLA-G in extravillous (intermediate) trophoblastic cells [1], there has been increased interest in studying HLA-G expression in neoplastic diseases based on an attractive hypothesis that cancer cells may likely use HLA-G expression to escape host immunosurveillance similar to what extravillous (intermediate) trophoblastic cells do in the maternal–fetal interface. Indeed, an increasing number of studies have reported HLA-G expression in several human cancers such as ovarian carcinoma, gastric carcinoma, coetaneous melanoma, hematopoietic tumors, endometrial carcinoma, renal cell carcinoma, lung carcinoma, mesothelioma, breast carcinoma and trophoblastic tumors [2–8]. Furthermore, secreted HLA-G (sHLA-G) has also been detected in some of these tumor types [9–11]. These studies provide cogent *in vivo* evidence demonstrating HLA-G expression in human tumor tissues and support the view that HLA-G may participate in tumor development by suppressing immune regulation within tumor microenvironment (see review article [2] for details). Besides the active basic research on the roles of HLA-G in immunomodulation, the translational-type studies that focus on the potential applications of HLA-G expression in human cancer diagnosis represent another research front. This review will summarize those recent studies of HLA-G expression in human cancer and, more importantly, will discuss the promise and challenges for applying the pattern of HLA-G expression to detect cancer in body fluids, to diagnose different cancer types, and to predict clinical outcome in cancer patients.

SHLA-G AS A BIOMARKER FOR CANCER DETECTION IN BODY FLUID

Because sHLA-G is a potential biomarker in body fluids, the measurement of sHLA-G levels in blood and effusion samples may have clinical values in differentiating ma-

From the Departments of Pathology, Oncology and Gynecology/Obstetrics, Johns Hopkins Medical Institutions, Baltimore, Maryland.

Address reprint requests to: Ie-Ming Shih, M.D., Ph.D., Department of Pathology, Johns Hopkins University School of Medicine, 1550 Orleans Street, Room 305, Baltimore, MD 21231; Tel: (410) 502-7774; Fax: (410) 502-7943; E-mail: ishih@jhmi.edu.

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lignant versus benign conditions. Rebmann *et al.* have elegantly applied enzyme-linked immunoassay (ELISA) to demonstrate an increased plasma sHLA-G level in patients with cutaneous melanoma, gliomas, breast, and ovarian carcinoma [10]. In hematopoietic neoplasms, as compared with control patients, the concentration of sHLA-G in blood increased in multiple myeloma [12], acute leukemia, especially in subtypes affecting monocytic and lymphoid lineages [13], in B-cell chronic lymphocytic leukemia, and non-Hodgkin B- and T-cell lymphomas [14]. These reports provide preliminary evidence that sHLA-G is a surrogate tumor marker that awaits further validation for potential clinical applications.

Ascites and pleural effusions are commonly associated with a variety of diseases including infection, inflammatory disorders, cardiac, liver and renal diseases as well as neoplastic diseases. Diagnosis is usually based on cytology by examining representative cells obtained from the effusion fluid, but the sensitivity of cytology has been estimated to be 60% at best [15]. The low sensitivity may be caused by small numbers of tumor cells in the ascites or the presence of a large amount of leukocytes, mesothelial cells, and blood that can obscure the malignant cells. For example, inflammation that is often associated with malignant ascites can result in reactive changes in mesothelial cells, making their morphologic distinction from carcinoma cells extremely difficult [15]. Therefore, molecular markers associated with malignant effusion would facilitate the diagnosis in this clinical setting.

Singer *et al.* applied a senstive ELISA to measure sHLA-G levels in the supernatant of peritoneal ascites from ovarian and breast carcinoma and benign controls [6]. In their study, the levels of sHLA-G were significantly higher in malignant ascites as compared with benign ascites. Interestingly, all but one malignant peritoneal ascites supernatant contained detectable sHLA-G including one specimen that had been missed on cytology. To detect ovarian and breast cancer in ascites using multiple cutoff values, the investigators used receiver operating characteristic (ROC) curves to evaluate the performance of sHLA-G. The area under the ROC curve was 0.95 in assessing sHLA-G levels as the diagnostic tool to detect ovarian and breast cancer.

Although sHLA-G may prove to be useful in assisting the diagnosis of malignant versus benign clinical conditions, it should be emphasized that body fluids from healthy individuals contain variable amount of sHLA-G probably derived from peripheral monocytes [16]. Thus a large number of cases should be assessed for ROC curve analysis to determine the optimal cutoff of sHLA-G for potential clinical applications. The other challenge to apply sHLA-G ELISA for cancer detection is to demonstrate a higher performance of sHLA-G than previously published or pre-existing markers. Several protein markers have been studied in cancer and they include CA125, tissue polypeptide specific antigen, soluble interleukin-2 receptor–α, soluble aminopeptidase N/CD13, α-fetoprotein, carcinoembryonic antigen, CA 19-9, CA 15-3, and several cytokines. Further studies should determine whether a combination of selected secreted biomarkers including sHLA-G may provide a better approach to increase both sensitivity and specificity than single marker alone for cancer detection in body fluids. It will be necessary to compare the performance of the sHLA-G ELISA and routine cytologic examination by testing a large number of cytology-negative but biopsy-positive samples. It will also be important to address how age, menopausal status, histologic grade, and other clinical parameters affect HLA-G levels in effusions.

ROLE OF HLA-G IN DIAGNOSIS OF HUMAN CANCER ON TISSUES

Diffuse pattern of HLA-G expression is associated with trophoblastic tumors and tumor-like lesions. As previously discussed, HLA-G was first identified as a molecule predominantly expressed in extravillous (intermediate) trophoblastic cells; therefore, it is likely that HLA-G expression should be detected in intermediate trophoblastic lesions. In fact, Singer *et al.* analyzed HLA-G immunoreactivity in human intermediate trophoblastic tumors including choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor and tumor-like lesions such as exaggerated placental site and placental site nodule. The researchers found that HLA-G immunoreactivity was detected in the majority of intermediate trophoblastic cells in all lesions. The diffuse staining pattern in virtually all intermediate trophoblastic lesions suggests that HLA-G immunoreactivity serves as a tissue marker to diagnose intermediate trophoblastic tumors and tumor-like lesions. As previously discussed, many human neoplastic diseases also express HLA-G; but the HLA-G immunoreactivity in those non-trophoblastic tumors is usually focal, with $<50\%$ of tumor cells being positive for HLA-G. For example, although HLA-G immunoreactivity is observed in more than 50% of the endometrial carcinomas that histologically may resemble trophoblastic tumors and tumor-like lesions, the majority ($>95\%$) of endometrial carcinoma cases exhibit positive HLA-G staining in less than 50% of tumor cells [17]. Therefore, diffuse HLA-G immunoreactivity within a tumor appears to be specific for intermediate trophoblastic cells in gestational trophoblastic diseases and can serve as a useful marker in the differential diagnosis of these lesions. The differential diagnosis between trophoblastic and non-trophoblastic lesions is