

HLA-G Proteins in Cancer: Do They Provide Tumor Cells with an Escape Mechanism?

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Abstract

Convincing clinical evidence indicates that the limited success of T-cell-based immunotherapy of malignant diseases is caused, at least in part, by the ability of malignant cells to escape from immune recognition and destruction. Among the multiple escape mechanisms identified, a major role is played by changes in the expression and/or function of HLA antigens expressed by tumor cells, because they may markedly affect tumor cell-host's immune system interactions. In this article, we review the data about the aberrant expression of the nonclassical HLA class I antigen HLA-G by tumor cells. Furthermore, we discuss the possible reasons for the conflicting information in the literature about HLA-G antigen expression by malignant cells. Lastly, in light of the well-documented immunotolerant function of HLA-G, we discuss the potential role of these antigens in the escape of tumor cells from immune recognition and destruction and in the clinical course of malignant diseases. (Cancer Res 2005; 65(22): 10139-44)

Introduction

Early studies have shown that the expression of the nonclassical HLA class I molecule HLA-G is restricted to the fetal-maternal interface on the extravillous cytotrophoblast (1). The presence of HLA-G at this immunologically privileged site was proposed to serve as a protection for the fetus from maternal allorecognition. This possibility is supported by the ability of HLA-G to suppress immune cell functions, such as natural killer (NK) cell-mediated and CTL-mediated cytolysis and T-cell proliferative response (for review, see ref. 2).

Over the past few years, the distribution of HLA-G in normal tissues has been found to be broader than originally reported: (a) HLA-G molecules have been detected in embryonic tissues from oocyte to blastocyst stage and then in invasive trophoblast, amniotic cells and fluid, endothelial cells from the chorionic villi, and erythroid cells in all organs sustaining primitive to definitive erythropoiesis; (b) in adult tissues, HLA-G antigens have been detected in thymic epithelial cells, in the epithelium, endothelium, and keratocytes from cornea, and in cells of the erythropoietic lineage from bone marrow (2). In addition, HLA-G antigens have been unexpectedly found in various types of malignant cells. The aberrant expression of HLA-G antigens by tumor cells has been suggested to be part of the strategies they use to escape from host's

immunosurveillance, in analogy with the role of HLA-G antigens in the escape of trophoblasts from maternal allorecognition (3).

Because of the current interest in defining the molecular nature of the immune escape mechanisms used by tumor cells, the potential role of HLA-G in these phenomena, and the significant amount of new data presented at the third international conference on HLA-G (4), in this article, we (a) describe the structural and functional characteristics of HLA-G antigens, (b) review the information about their expression in malignant cells, and (c) discuss the potential role of HLA-G antigens in tumor cell escape mechanisms and in the clinical course of malignant diseases.

Structural and Functional Characteristics of HLA-G Molecules

Besides its limited polymorphism with the expression of 15 alleles (including 1 null allele; for review, see ref. 2), a distinct feature of HLA-G is its possible expression in seven isoforms. Alternative splicing of the primary HLA-G transcript leads to the synthesis of the four membrane-bound HLA-G1, HLA-G2, HLA-G3, and HLA-G4 isoforms as well as of the three soluble HLA-G5, HLA-G6, and HLA-G7 isoforms.

HLA-G antigens play a key role in the establishment and maintenance of immune tolerance by inhibiting the function(s) of immunocompetent cells (2). Such inhibitory effects are mediated by the direct binding of HLA-G to inhibitory receptors [i.e., immunoglobulin-like transcript (ILT)-2 (CD85j) expressed by lymphoid and myelomonocytic cells and ILT-4 (CD85d) expressed by dendritic cells, macrophages, and monocytes]. Although both receptors have other HLA class I ligands, they have been reported to have the highest affinity for HLA-G (2). Furthermore, KIR2DL4/p49 (CD158d) has been described as a HLA-G-specific receptor expressed by all NK cells. Therefore, the HLA-G protein can, through these receptors, directly interact with B, NK, T, and antigen-presenting cells and exert its immunotolerant functions at different stages of the immune response (i.e., differentiation, proliferation, cytolysis, and cytokine secretion). HLA-G may also exert its immunosuppressive effect through an indirect pathway by allowing the cell surface expression of HLA-E, another nonclassical HLA class I molecule. The latter molecule inhibits both NK and T cells by interacting with the inhibitory receptor CD94/NKG2A (5). It is of note that the above-described functions are not exclusive to HLA-G1 protein but are shared with the HLA-G2, HLA-G3, and HLA-G4 protein isoforms (2) and with the soluble HLA-G5 protein. The latter antigen can also induce apoptosis of activated CD8⁺ T cells and NK cells by binding to CD8 and triggering a Fas/Fas ligand (FasL)-dependent pathway (2). HLA-G5 may exert its inhibitory effects also through a feedback mechanism, because it inhibits the proliferative response of alloreactive CD4⁺ T cells that secrete it (6). Whereas membrane-bound HLA-G proteins can act

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locally where they are expressed, soluble HLA-G5 may exert functions in the nearby environment of its site of origin and at distant sites because of distribution via the circulation system.

Cytokine-mediated effects represent another molecular mechanism by which HLA-G can exert immunosuppression. In this regard, HLA-G has been also shown to modulate the release of cytokines from peripheral blood and decidual mononuclear cells toward Th2 profile. HLA-G protein may in turn be induced by several cytokines, such as granulocyte macrophage colony-stimulating factor, interleukin (IL)-10, IFNs, and leukemia inhibitory factor (for review, see ref. 2). Interestingly, the presence of IL-10, which is secreted by cutaneous lymphoma cells and is able *in vitro* to induce HLA-G expression, is correlated with HLA-G protein expression in these tumors (7). Because of their immunosuppressive properties (2), IL-10 and HLA-G might both contribute to the evasion of malignant cells from immunosurveillance and favor the transition from low-grade to high-grade lymphoma.

Both IFN- β and IFN- γ have been shown to enhance HLA-G cell surface expression *in vitro* (2). Therefore, one potential side effect of the administration of IFNs for immunotherapy of malignant diseases is represented by the up-regulation of HLA-G expression at the tumor site. This phenotypic change may confer immunoprotection to tumor cells, thus facilitating their spreading in the host (8, 9). In this regard, an association has been described between lack of clinical response to therapy with high-dose IFN- α 2b and HLA-G expression in melanoma lesions (9). This association suggests that screening of melanoma lesions for HLA-G expression may represent a useful strategy to identify patients with melanoma who are likely to benefit from therapy with IFN- α .

HLA-G antigens have also been reported to be expressed by immune cells infiltrating tumor sites and in peripheral blood from cancer patients (7, 8, 10–13). In view of the expression of the HLA-G-binding inhibitory receptors (ILT-2 and ILT-4) on B and T lymphocytes as well as on dendritic cells and monocytes/macrophages, it remains to be determined whether HLA-G immunoreactivity in these cells is due to endogenous expression of HLA-G or to the binding of soluble HLA-G molecules to these receptors. These studies will benefit from the recently developed antibodies, which react specifically with soluble HLA-G proteins (4). Notably, antigen-presenting cells, such as dendritic cells and monocytes/macrophages, are able to endogenously express HLA-G upon *in vitro* cytokine treatment, such as IFN and IL-10, supporting an up-regulation of HLA-G expression in these cells within a tumor microenvironment containing such cytokines (2). Therefore, HLA-G antigens expressed by such antigen-presenting cells may block the triggering of a patient's immune response to his own tumor. This possibility is supported by the ability of HLA-G tetramers to inhibit the maturation of immature dendritic cells into mature and stimulatory dendritic cells via binding to the ILT-4 receptor in humans and to the paired immunoglobulin-like receptor- β receptor in mice (14). Moreover, HLA-G-positive antigen-presenting cells have been shown recently to inhibit CD4⁺ T cells in response to antigen stimulation and to trigger their differentiation into suppressor cells (4).

HLA-G Expression in Malignant Lesions

The scientific community's interest in assessing the potential role of HLA-G in tumor cell escape from immunosurveillance has stimulated the analysis of HLA-G expression in malignant lesions. During the last 5 years, >1,000 malignant lesions have been tested

for HLA-G expression. The assay systems used include immunohistochemical staining of frozen and formalin-fixed, paraffin-embedded tissue sections with monoclonal antibody (mAb) and Western blotting analysis of tumor lesion lysates with mAb. The HLA-G1 and HLA-G5 antigen-specific mAb 87G and the HLA-G-specific mAb 4H84 have been used as probes in most of the studies. The specificity of both mAbs has been validated during international workshops on HLA-G/HLA-E. The mAb 4H84 is not commercially available, whereas the mAb 87G has become recently commercially available.

The information in the literature about HLA-G expression in various types of tumors, which is summarized in Fig. 1, deserves several comments. First, 16 types of tumors have been analyzed. Seven are of ectodermic origin, six of mesodermic origin, and three of entodermic origin. Second, different numbers of lesions have been analyzed in each type of malignancy. The largest number of samples has been analyzed in leukemia (15). Among solid tumors, the number of lesions analyzed ranges from ~10 in basal cell carcinoma to >100 in melanoma (9, 16, 17) and in

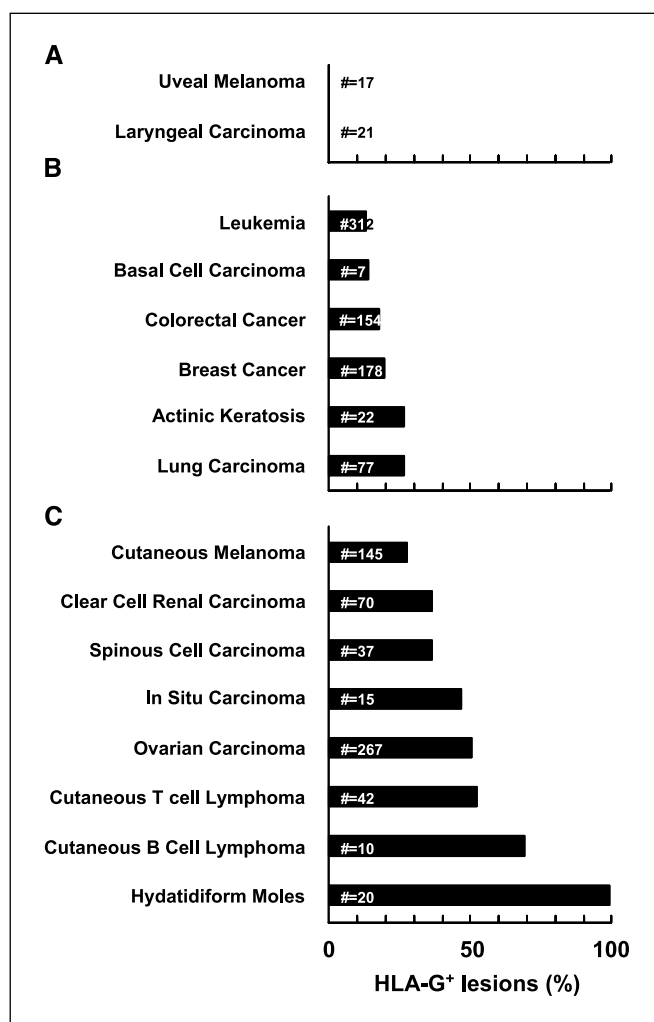


Figure 1. HLA-G expression in tumor lesions. For each tumor type, the total number of lesions indicated as # and the corresponding percentage of HLA-G-positive lesions were determined by taking into account all the studies published up to now. Malignant tumors were divided into three groups: those without detectable HLA-G expression (A), those with <28% of lesions expressing HLA-G (B), and those with >37% of lesions expressing HLA-G (C).

breast (12, 18), colorectal, and ovarian (18, 19) carcinomas. Third, there are marked differences in the frequency of HLA-G expression in tumors, as it ranges from 100% in hydatiform moles most likely because of its placental origin to 0% in uveal melanoma and laryngeal carcinoma. Using the frequency of HLA-G expression as a criterion, tumors can be divided into three groups: (a) those in which HLA-G has not been detected, such as uveal melanoma and laryngeal carcinoma; (b) those in which HLA-G has been detected in at least 37% of the lesions tested, such as clear cell renal carcinoma (20, 21), ovarian carcinoma (18, 19), and cutaneous lymphoma (7, 22); and (c) those in which HLA-G has been detected in <28% of the lesions tested, such as cutaneous melanoma (16, 17), lung carcinoma (10, 11), and breast carcinoma (12, 18).

The different results obtained with various types of malignancies may reflect differences in the biology of the various types of tumors analyzed, in the patient populations tested, and/or in the sensitivity of the methods used to detect HLA-G antigens in malignant lesions. Furthermore, the regulation of *HLA-G* gene expression may differ among different tumor types and may be influenced by the tumor microenvironment as well as by the pathogenesis underlying the malignant transformation of cells. The latter possibility is supported by the marked difference in HLA-G expression between cutaneous and hematologic lymphomas. Lastly, the tumor cell type analyzed may also influence the frequency of HLA-G expression as exemplified by renal cell carcinoma. HLA-G is not expressed in all types of renal epithelial cell tumors but is restricted to clear cell carcinoma (20, 23).

The expression of HLA-G is associated with malignant transformation because HLA-G has never been detected in adjacent normal tissues. This point is illustrated by the analysis of sequential biopsies of healthy skin, primary cutaneous tumor, lymph node metastasis, and a tumor regression site within the skin primary tumor all obtained from a patient with melanoma. HLA-G protein was expressed in both primary and metastatic tumor sites but was not detected in both healthy skin and tumor regression site in which an efficient immune response may occur in the absence of HLA-G (16). In agreement with this observation, in transplant patients who develop both benign and malignant cutaneous lesions at distinct sites, activation of HLA-G expression is restricted to the malignant site leading to the conclusion that HLA-G expression is associated with tumor development (13).

Heterogeneity in HLA-G expression in tumor lesions is also observed among patients within a tumor type as well as within individual lesions. For instance, in breast carcinoma and melanoma lesions, HLA-G has been detected either in the infiltrating cells or in the tumor cells depending on the patient (refs. 12, 17; Fig. 2). In addition, the number of HLA-G-expressing cells varies among patients within a type of tumor as described in renal cell carcinoma (23) and leukemia (15). In most tumors, HLA-G is focally expressed. Regarding the correlation between HLA-G expression in tumor lesions and the stage or grade of the tumor, results vary according to the tumor type analyzed as exemplified by the correlation found in lung carcinoma lesions (10) but not in renal cell carcinoma lesions (23).

Increased Soluble HLA-G Levels in Serum and Ascites from Patients with Malignancies

Like other types of HLA class I antigens, soluble HLA-G antigens are present in serum. Their level, which has been measured using

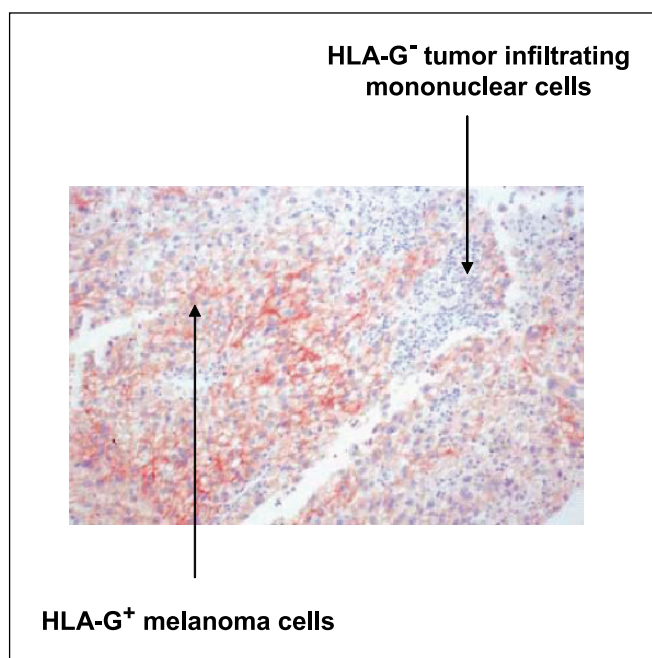


Figure 2. Intratumor variation in HLA-G expression. A formalin-fixed, paraffin-embedded primary melanoma lesion section was stained with the HLA-G-specific mAb 4H84. HLA-G is expressed with a heterogeneous pattern in melanoma cells and is not detectable in infiltrating mononuclear cells.

various antibody-based assays, is ~20 ng/mL in healthy individuals (24, 25). Regarding the source of soluble HLA-G *in vivo*, both immune cells and tumor cells may produce it. For instance, HLA-G antigens are expressed in peripheral blood monocytes from melanoma patients with elevated soluble HLA-G serum level (8). Furthermore, T cells, dendritic cells, and monocytes/macrophages are able to secrete soluble HLA-G molecules *in vitro* (2, 25). Serum HLA-G antigens, which are derived from the release of membrane-bound HLA-G isoforms, like HLA-G1 (HLA-G1s for HLA-G1 shedding), and from the secretion of soluble HLA-G isoforms, like HLA-G5, may affect antitumor immune response both locally at the tumor site and systemically by distribution via the circulation. Soluble HLA-G plasma levels are significantly increased in patients with malignant melanoma, glioma, and breast and ovarian carcinoma (25) as well as in those with lymphoproliferative disorders (26). Notably, in patients suffering from glioblastoma multiforme, their survival after tumor diagnosis is inversely correlated with serum HLA-G level. In melanoma patients, serum HLA-G level is correlated with advanced disease stage and with tumor load (8). These observations parallel results in transplanted patients in whom increased soluble HLA-G serum level is associated with an improved allograft acceptance (24, 27). Taken together, these findings suggest that an increased soluble HLA-G level in biological fluids is associated with a down-modulation of the immune response.

In agreement with this possibility, soluble HLA-G levels are increased in malignant ascites from ovarian and breast carcinomas compared with benign ones. This finding leads to the possibility that HLA-G may be a potential tumor marker and that measurement of soluble HLA-G constitutes a useful way in addition to cytology for the differential diagnosis of malignant versus benign ascites (18). Furthermore, HLA-G expression in

cancer cells from malignant effusions has been proposed as a possible marker of tumor susceptibility to chemotherapy in patients with advanced-stage ovarian carcinoma (19).

Whether a relationship exists between HLA-G expression in the tumor lesion and its serum level remains still to be determined. To the best of our knowledge, only one type of patient's sample (biopsy or serum) has been analyzed in all studies, except one. In this study on leukemia patients, plasma HLA-G levels tended to be higher in patients with HLA-G-positive leukemias than in those with HLA-G-negative leukemias (15). In patients with breast and ovarian carcinoma, the frequency of HLA-G expression in tumors as measured by immunohistochemistry is lower than that in malignant ascites as measured with an antibody-based ELISA (18).

***In vitro* HLA-G Expression by Cell Lines**

More than 200 cell lines have been studied up to now for their expression of HLA-G on the cell surface by flow cytometry or in the cytoplasm by immunocytochemistry and Western blot analysis. The results in the literature about HLA-G expression by cell lines are more concordant than those derived from the analysis of surgically removed tumor lesions. It is a general experience that, with the exception of choriocarcinoma cell lines, HLA-G is expressed in a very low number of cell lines in long-term culture. As discussed already with malignant lesions, cell lines can be divided into three groups: (a) in some types of cell lines, such as breast carcinoma cell lines, HLA-G has never been detected; (b) in melanoma cell lines (28) and renal cell carcinoma cell lines (21), HLA-G has been detected only in a minority of cell lines; and (c) in cell lines of neuroectodermal origin, HLA-G is expressed in the large majority of cell lines (29). Interestingly, incubation with IFN (α , β , and γ) enhances HLA-G1 cell surface expression on cell lines that express this antigen (21). More relevant, incubation with IFN- γ up-regulates *HLA-G* gene transcription and induces HLA-G1 cell surface expression on cell lines without detectable expression of this antigen (29).

The discrepancy between the *in vitro* data and the results obtained by testing surgically removed lesions may reflect the lack in tissue culture medium of factors that induce and maintain HLA-G expression in the tumor microenvironment. In agreement with this possibility is our recent finding that HLA-G was lost after several passages in culture by a melanoma cell line that had a high HLA-G expression when established from a HLA-G-positive melanoma biopsy (ref. 30; Fig. 3). Similarly, HLA-G expression was down-regulated or even lost following a few passages in culture by short-term ovarian carcinoma cell lines and renal carcinoma cell lines, whereas classical HLA class I antigen expression was not affected under these conditions (20, 31). These results should be kept in mind when analyzing the results obtained with cell lines because most of them have been cultured *in vitro* for many years.

The factors that are involved in ectopic activation of *HLA-G* gene transcription and protein expression in tumor cells remain to be defined. In this regard, a model has recently been proposed that may account for the preferential *in vivo* HLA-G expression in tumor cells (32). When malignant transformation occurs, tumor cells are likely to be exposed to a variety of cytokines and of stress factors from the tumor microenvironment and to undergo epigenetic changes, such as DNA hypomethylation and histone acetylation, which activate the *HLA-G* gene (33). The appearance

of HLA-G on tumor cells may then down-regulate cellular immune responses and increase their chances of survival. However, on adaptation to tissue culture, tumor cells are not exposed to the same stressful conditions. Consequently, methylation of the HLA-G promoter and silencing of the gene may occur.

Functional Significance of HLA-G Expression by Tumor Cells

Like trophoblasts that do not express classical HLA class I antigens (1), allowing their escape from CTL killing, human tumor cells frequently down-regulate or lack classical HLA class I molecule expression. These defects, which may be associated with loss of heterozygosity (34), are often associated with a poor clinical course of the disease, although such classical HLA class I-deficient tumor cells should be susceptible to NK cell-mediated lysis. This finding has led to the hypothesis that malignant cells are protected from NK cell-mediated killing by HLA-G antigens.

In vitro studies have shown that glioma, melanoma, and renal carcinoma cell lines that express endogenous HLA-G isoforms as well as melanoma cell lines that express HLA-G following transfection with HLA-G cDNA are protected from lysis by alloreactive NK and lymphokine-activated killer cells and/or by antigen-specific CD8⁺ T cells (20, 28, 29). This protective effect can be reversed by blocking HLA-G1 with HLA-G1-specific mAb.

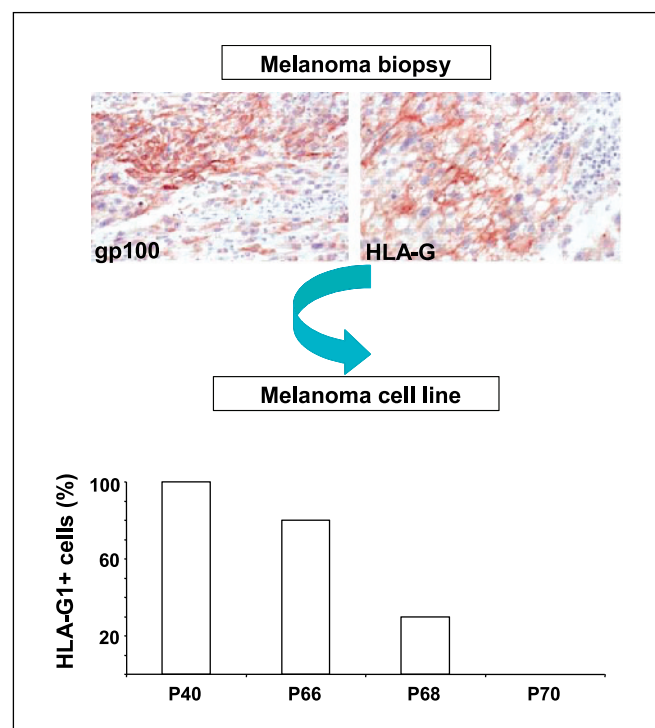


Figure 3. Loss of HLA-G cell surface expression during long-term culture of a melanoma cell line derived from a surgically removed primary melanoma lesion. A formalin-fixed, paraffin-embedded primary melanoma lesion section was positively stained by gp100-specific mAb HMB45 and by HLA-G-specific mAb 4H84. A melanoma cell line was derived *in vitro* from the tumor lesion. The primary culture cell line expressed high levels of HLA-G1 that was maintained until passage 40 (P40). HLA-G cell surface expression started to decrease at passage 66 (P66) and became undetectable at passage 70 (P70). HLA-G expression was not detectable on the long-term propagated cell line. HLA-G1 cell surface expression was assessed by flow cytometry analysis of cells stained with HLA-G1-specific mAb MEM-G/9.

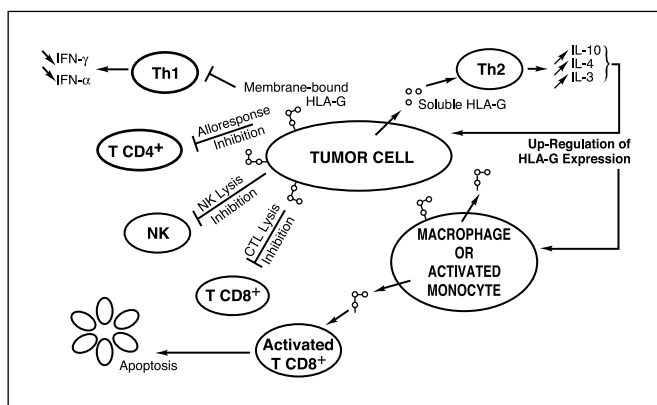


Figure 4. Schematic representation of potential inhibitory effect of HLA-G expressed by tumor cells and/or immune infiltrating cells on tumor antigen-specific immune response. At a tumor site, HLA-G can be expressed on cell membrane and/or released in soluble form from tumor cells and/or infiltrating cells, such as macrophages. Both membrane-bound and soluble HLA-G proteins inhibit the lytic activity and the proliferative response of effector cells belonging to innate immunity, such as NK cells, and to adaptive immunity, such as CD4⁺ and CD8⁺ T cells. In addition, soluble HLA-G produced by tumor cells and/or by immune cells induces apoptosis of activated CD8⁺ T cells by binding to CD8 and by triggering a Fas/FasL-dependent pathway. Lastly, whereas membrane-bound HLA-G down-regulates IFN secretion by T cells, soluble HLA-G stimulates the release of IL-3, IL-4, and IL-10. Thus, HLA-G induces Th2 cytokine profile that in turn up-regulates HLA-G expression at the tumor site. These multiple immunosuppressive effects are mediated by the binding of HLA-G to several receptors, including CD8, KIR2DL4/p49, ILT-2, and ILT-4.

In addition, the expression of HLA-G in short-term ovarian carcinoma cell lines has resulted in resistance of these cells to lysis by peptide-specific and allospecific CD8⁺ T cells. This effect was not directly due to HLA-G but reflects its ability to induce HLA-E expression on tumor cells (31). Finally, exosomes secreted by HLA-G-positive melanoma cells have been found to bear HLA-G on their membrane (35).

Ligands that activate NK cell-mediated tumor cytotoxicity, such as the stress-inducible molecule MICA, have been reported to be selectively switched on in various tumors of both epithelial and nonepithelial origins (36). Analysis of the balance between the activating signal delivered by MICA and the inhibitory signal generated by HLA-G1 on NK cell-mediated lysis of a melanoma cell

line has showed that HLA-G1 counteracts the triggering signal of MICA (37). This finding suggests that *in vivo* the overexpression of inhibitory ligands, such as HLA-G, by tumor cells may bypass activating signal(s) mediated by MICA, thereby favoring tumor progression (38).

Conclusion

During the last few years, the HLA-G field has evolved from being a topic restricted to reproduction to a very broad topic that spans from oncology to transplantation to autoimmunity as illustrated by the many presentations at the last international conference on HLA-G (4). Consequently, in a short time, a significant amount of information has been accumulated, particularly on HLA-G in tumors.

Based on the information we have reviewed, we propose that HLA-G whose engagement generates inhibitory signals in various immune cells may represent a mechanism used by tumor cells to escape from immunosurveillance (Fig. 4). Testing of the validity of this possibility will benefit from the development of an animal model system. This model would also provide the opportunity to test the affect on the clinical course of malignant diseases of strategies, which interfere with HLA-G expression and/or function. Furthermore, the relationship found between HLA-G expression in melanoma lesions and clinical response to therapy with IFN- α (9) suggests that HLA-G expression in tumor lesions should be taken into account in the evaluation of the outcome of immunology-based therapies of malignant diseases. Lastly, the recently described association of HLA-G expression in B-cell chronic leukemia with a strong immunodeficiency and a poor clinical evolution raises the possibility that HLA-G expression may represent a useful prognostic marker in at least some malignant diseases (15).

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References

- Kovats S, Main EK, Librach C, Stubblebine M, Fisher SJ, DeMars R. A class I antigen, HLA-G, expressed in human trophoblasts. *Science* 1990;248:220-3.
- Carosella ED, Moreau P, Le Maoult J, Le Discorde M, Dausset J, Rouas-Freiss N. HLA-G molecules: from maternal-fetal tolerance to tissue acceptance. *Adv Immunol* 2003;81:199-252.
- Chang CC, Campoli M, Ferrone S. HLA class I antigen expression in malignant cells: why does it not always correlate with CTL-mediated lysis? *Curr Opin Immunol* 2004;16:644-50.
- LeMaoult J, Le Discorde M, Rouas-Freiss N, et al. Biology and functions of human leukocyte antigen-G in health and sickness. *Tissue Antigens* 2003;62:273-84.
- Braud VM, Allan DS, O'Callaghan CA, et al. HLA-E binds to natural killer cell CD94/NKG2A, B and C. *Nature* 1998;391:795-9.
- Lila N, Rouas-Freiss N, Dausset J, Carpentier A, Carosella ED. Soluble HLA-G protein secreted by allo-specific CD4⁺ T cells suppresses the allo-proliferative response: a CD4⁺ T cell regulatory mechanism. *Proc Natl Acad Sci U S A* 2001;98:12150-5.
- Urošević M, Willers J, Mueller B, Kempf W, Burg G, Dummer R. HLA-G protein up-regulation in primary cutaneous lymphomas is associated with interleukin-10 expression in large cell T-cell lymphomas and indolent B-cell lymphomas. *Blood* 2002;99:609-17.
- Uğurel S, Rebmann V, Ferrone S, Tilgen W, Grosse-Wilde H, Reinhold U. Soluble human leukocyte antigen-G serum level is elevated in melanoma patients and is further increased by interferon- α immunotherapy. *Cancer* 2001;92:369-76.
- Wagner SN, Rebmann V, Willers CP, Grosse-Wilde H, Goos M. Expression analysis of classic and non classic HLA molecules before interferon alpha-2b treatment of melanoma. *Lancet* 2000;356:220-1.
- Urošević M, Kurrer MO, Kamarashev J, et al. Human leukocyte antigen G up-regulation in lung cancer associates with high-grade histology, human leukocyte antigen class I loss and interleukin-10 production. *Am J Pathol* 2001;159:817-24.
- Pangault C, Le Fric G, Caulet-Maugendre S, et al. Lung macrophages and dendritic cells express HLA-G molecules in pulmonary diseases. *Hum Immunol* 2002; 63:83-90.
- Lefebvre S, Antoine M, Uzan S, et al. Specific activation of the non-classical class I histocompatibility HLA-G antigen and expression of the ILT2 inhibitory receptor in human breast cancer. *J Pathol* 2002;196:266-74.
- Aractingi S, Kanitakis J, Euvrard S, Le Danff C, Carosella ED. Selective expression of HLA-G in malignant and premalignant skin specimens in kidney transplant recipients. *Int J Cancer* 2003;106:232-5.
- Ristich V, Liang S, Zhang W, Wu J, Horuzsko A. Tolerization of dendritic cells by HLA-G. *Eur J Immunol* 2005;35:1133-42.
- Nuckel H, Rebmann V, Durig J, Duhrsen U, Grosse-Wilde H. HLA-G expression is associated with an unfavorable outcome and immunodeficiency in chronic lymphocytic leukemia. *Blood* 2005;105:1694-8.
- Paul P, Cabestre FA, Le Gal FA, et al. Heterogeneity of HLA-G gene transcription and protein expression in malignant melanoma biopsies. *Cancer Res* 1999;59: 1954-60.

17. Ibrahim el C, Aractingi S, Allory Y, et al. Analysis of HLA antigen expression in benign and malignant melanocytic lesions reveals that upregulation of HLA-G expression correlates with malignant transformation, high inflammatory infiltration and HLA-A1 genotype. *Int J Cancer* 2004;108:243-50.
18. Singer G, Rebmann V, Chen YC, et al. HLA-G is a potential tumor marker in malignant ascites. *Clin Cancer Res* 2003;9:4460-4.
19. Davidson B, Elstrand MB, McMaster MT, et al. HLA-G expression in effusions is a possible marker of tumor susceptibility to chemotherapy in ovarian carcinoma. *Gynecol Oncol* 2005;96:42-7.
20. Bukur J, Rebmann V, Grosse-Wilde H, et al. Functional role of human leukocyte antigen-G up-regulation in renal cell carcinoma. *Cancer Res* 2003;63:4107-11.
21. Ibrahim EC, Guerra N, Lacombe MJ, et al. Tumor-specific up-regulation of the nonclassical class I HLA-G antigen expression in renal carcinoma. *Cancer Res* 2001;61:6838-45.
22. Urosevic M, Kamarashev J, Burg G, Dummer R. Primary cutaneous CD8⁺ and CD56⁺ T-cell lymphomas express HLA-G and killer-cell inhibitory ligand, ILT2. *Blood* 2004;103:1796-8.
23. Ibrahim el C, Allory Y, Commo F, Gattegno B, Callard P, Paul P. Altered pattern of major histocompatibility complex expression in renal carcinoma: tumor-specific expression of the nonclassical human leukocyte antigen-G molecule is restricted to clear cell carcinoma while up-regulation of other major histocompatibility complex antigens is primarily distributed in all subtypes of renal carcinoma. *Am J Pathol* 2003;162:501-8.
24. Creput C, Durrbach A, Menier C, et al. Human leukocyte antigen-G (HLA-G) expression in biliary epithelial cells is associated with allograft acceptance in liver-kidney transplantation. *J Hepatol* 2003;39:587-94.
25. Rebmann V, Regel J, Stolke D, Grosse-Wilde H. Secretion of sHLA-G molecules in malignancies. *Semin Cancer Biol* 2003;13:371-7.
26. Amiot L, Le Fric G, Sebti Y, et al. HLA-G and lymphoproliferative disorders. *Semin Cancer Biol* 2003;13:379-85.
27. Lila N, Amrein C, Guillemain R, et al. Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. *Circulation* 2002;105:1949-54.
28. Paul P, Rouas-Freiss N, Khalil-Daher I, et al. HLA-G expression in melanoma: a way for tumor cells to escape from immunosurveillance. *Proc Natl Acad Sci U S A* 1998;95:4510-5.
29. Wiendl H, Mitsdoerffer M, Hofmeister V, et al. A functional role of HLA-G expression in human gliomas: an alternative strategy of immune escape. *J Immunol* 2002;168:4772-80.
30. Rouas-Freiss N, Bruel S, Menier C, Marcou C, Moreau P, Carosella ED. Switch of HLA-G alternative splicing in a melanoma cell line causes loss of HLA-G1 expression and sensitivity to NK lysis. *Int J Cancer* 2005;117:114-22.
31. Malmberg KJ, Levitsky V, Norell H, et al. IFN- γ protects short-term ovarian carcinoma cell lines from CTL lysis via a CD94/NKG2A-dependent mechanism. *J Clin Invest* 2002;110:1515-23.
32. Chang CC, Ferrone S. HLA-G in melanoma: can the current controversies be solved? *Semin Cancer Biol* 2003;13:361-9.
33. Moreau P, Mouillot G, Rousseau P, Marcou C, Dausset J, Carosella ED. HLA-G gene repression is reversed by demethylation. *Proc Natl Acad Sci U S A* 2003;100:1191-6.
34. Ferrone S, Marincola F. Loss of HLA class I antigens by melanoma cells: molecular mechanisms, functional significance and clinical relevance. *Immunol Today* 1995;16:487-94.
35. Riteau B, Faure F, Menier C, et al. Exosomes bearing HLA-G are released by melanoma cells. *Hum Immunol* 2003;64:1064-72.
36. Groh V, Rhinehart R, Secrist H, Bauer S, Grabstein KH, Spies T. Broad tumor-associated expression and recognition by tumor-derived $\gamma\delta$ T cells of MICA and MICB. *Proc Natl Acad Sci U S A* 1999;96:6879-84.
37. Menier C, Riteau B, Carosella ED, Rouas-Freiss N. MICA triggering signal for NK cell tumor lysis is counteracted by HLA-G1-mediated inhibitory signal. *Int J Cancer* 2002;100:63-70.
38. Seliger B, Abken H, Ferrone S. HLA-G and MIC expression in tumors and their role in anti-tumor immunity. *Trends Immunol* 2003;24:82-7.