Molecular Pathways

Human leukocyte antigen G (HLA-G)

Giuseppe Curigliano, Carmen Criscitiello, Lucia Gelao and Aron Goldhirsch

Division of Early Drug Development for Innovative Therapies, Istituto Europeo di Oncologia, Via Ripamonti 435, 20133 Milano, Italia

Correspondence to:

Giuseppe Curigliano, MD, PhD
Division of Early Drug Development for Innovative Therapies,
Istituto Europeo di Oncologia, Via Ripamonti 435, 20133 Milano, Italia

E-mail: giuseppe.curigliano@ieo.it

URL: http://www.ieo.it

Phone: +39-02-57489788

Fax: +39-02-57489581

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Abstract

Human leukocyte antigen G (HLA-G) is a non-classical major histocompatibility complex (MHC) class I molecule that exerts important tolerogenic functions. Its main physiological expression occurs in placenta where participates in the maternal tolerance toward the fetus. HLA-G expression was found in embryonic tissues, in adult immune privileged organs, and in cells of the hematopoietic lineage. It is expressed in various types of primary solid (melanoma, head and neck, lung, urogenital, gastrointestinal and breast cancers) and hematological malignancies (acute leukemia, lymphomas) and metastases. HLA-G ectopic expression is observed in cancer suggesting that its expression is one strategy used by tumor cells to escape immune surveillance. In this review, we will focus on HLA-G expression in cancers and its association with the prognosis. We will highlight the underlying molecular mechanisms of impaired HLA-G expression, the immune tolerant function of HLA-G in tumors, and the potential diagnostic use of membrane-bound and soluble HLA-G as a biomarker to identify tumors and to monitor disease stage. Since HLA-G is a potent immuno-inhibitory molecule, its blockade remains an attractive therapeutic strategy against cancer. Elimination of HLA-G-expressing cancer cells would be important in the efficacy of anti-cancer therapies.
Background

Structure and physiology of HLA-G

The physiological HLA-G expression is restricted to fetal tissues such as amniotic cells, erythroid precursors and cytotrophoblasts, and, in adults, to immune-privileged organs including cornea, thymus, pancreatic islets, endothelial cell precursors and erythroblasts (1). Dendritic cells [(DC) also known as antigen presenting cells] and macrophages (specialized in removal of dying or dead cells and cellular debris) can also express HLA-G. HLA-G expression is induced in cancers, conditions as transplantation, and diseases as multiple sclerosis, inflammatory diseases and viral infections. HLA-G can generate by alternative splicing seven alternative mRNAs encoding four membrane-bound (HLA-G1–HLA-G4) and three secreted isoforms (sHLA-G; HLA-G5–HLA-G7), which lack the transmembrane domains. HLAG1 can also be released into the medium by proteolytic cleavage as shed HLA-G1 (sHLAG1) (2). Specifically the primary transcript produced by transcription of the HLA-G gene may yield 7 protein isoforms that all possess the $\alpha_1$ domain (2). Four isoforms: HLA-G1, -G2, -G3 and -G4 have transmembrane and cytoplasmic domains and are therefore membrane-bound. HLA-G1 contains the three globular domains, HLA-G2 contains the $\alpha_1$ and $\alpha_3$ domains, HLA-G4 contains the $\alpha_1$ and $\alpha_2$ domains, and HLA-G3 is constituted of the $\alpha_1$ domain only. The mRNAs that encode for the HLA-G5 and -G6 isoforms retain the intron 4 (2). Due to the existence of an intron-4-encoded stop codon, translation is prematurely interrupted and the transmembrane domain and cytoplasmic domains are not translated. As a result, these isoforms are secreted proteins with a small tail after the $\alpha_3$ domain, corresponding to the translated part of intron 4. HLA-G5 is the soluble counterpart of HLA-G1 and has the three globular domains, whereas HLA-G6 is the soluble counterpart of HLA-G2 and contains only the $\alpha_1$ and $\alpha_3$ domains. Most of the available information concerns the HLA-G1 molecule and its soluble...
counterpart HLA-G5. They are composed of the heavy chain (HC) consisting of three globular domains (α1, α2, α3) noncovalently bound to β2-microglobulin (β2-m) and a nonapeptide (1). HLA-G induces tolerance by inhibiting different immune-competent cells. Its inhibitory effect is mediated by binding of both soluble and membrane-bound HLA-G to inhibitory receptors, such as the immunoglobulin-like transcript (ILT) receptor 2 (CD85j; LILRB1) present on lymphoid and myelo-monocytic cells and ILT-4 (CD85d; LILRB2) expressed by DC, macrophages and monocytes (3-5). The killer cell immunoglobulin-like receptor (KIR) 2DL4/p49 (CD158d) expressed by natural killer (NK) cells also represents an HLA-G-specific receptor (6). HLA-G directly interacts with different immune cell subpopulations and induces the maintenance of tolerance at different stages of the immune response, e.g., differentiation, proliferation, cytolytic and cytokine secretion. HLA-G antigens can be expressed on tumor-infiltrating immune cells (TIL) as well as in peripheral blood from cancer patients (7–9). Due to its tolerogenic effects HLA-G may impair patients' immune response against tumor. HLA-G+ antigen presenting cell (APC) complex can inhibit the function of CD4+ T cells and induce their differentiation into regulatory T cells (Treg) (10). Treg, formerly known as suppressor T cells, are a subpopulation of T cells which modulate the immune system, maintain tolerance to self-antigens, and abrogate autoimmune disease (Figure 1).

By indirect mechanisms, HLA-G can exert immune suppressive activity, specifically it can express the non-classical HLA class I molecule HLA-E, which directly binds peptides derived from HLA-G. This molecule can interact with the inhibitory receptor CD94/NKG2A resulting in the inhibition of NK and T cell reactivity (11).

**HLA-G immune function**

HLA-G receptors immune inhibition mechanisms can be organized into: (a) the direct immuno-inhibitory functions through blocking effector cells, (b) the indirect immuno-inhibitory functions
through regulatory cell generation, and (c) the other functions of HLA-G that have immuno-
inhibitory consequences.

**Inhibition of effector cells**

HLA-G is primarily expressed at the maternal-fetal interface by cytotrophoblasts cells that do not express HLA class I molecules, apart from HLA-C at low levels and HLA-G. In this physiological context HLA-G1 isoform inhibits the cytolytic function of uterine and peripheral NK cells (12, 13). Trophoblast cells are protected by HLA-G from cytolysis by decidual and peripheral NK cells of semi-allogeneic or allogeneic origin. As reported in figure 2, box A, HLA-G may interact with inhibitory receptors at the surface of the NK cells, leading to their functional inhibition. It was also demonstrated that through direct interaction with ILT2 or ILT4 inhibitory receptors, HLA-G inhibited CTLs (Figure 2, Box B).

**Modulation of regulatory/suppressor cells**

HLA-G possessed “long-term” tolerogenic functions as well, through the generation of regulatory/suppressor cells. Regulatory cells are immune effectors that are critically involved in promoting and maintaining immune tolerance because they can inhibit the reactivity of other effectors. The capability of HLA-G to induce regulatory T cells was investigated, and it was demonstrated that CD4+ and CD8+ T cells that had been stimulated in the presence of HLA-G not only lost their capability to respond to antigenic stimulation, but also differentiated into regulatory T cells capable of inhibiting the reactivity of other T cells (10). Figure 2, Box C summarizes this mechanism of immune-inhibition. HLA-G-induced regulatory T cells required HLA-G for their generation but not for their function (14) and, once generated, HLA-G-induced regulatory cells no longer required HLA-G to be tolerogenic (14). Activated NK and T cells – and to a lesser extent monocytes - may also temporarily behave as regulatory suppressor cells and inhibit immune...
responses through HLA-G after they have acquired HLA-G-containing membranes from cells in their vicinity by the mechanism of trogocytosis (15) (Figure 2, Box D). Trogocytosis is the transfer of plasma membrane and anchored proteins during cell-to-cell contact. A thorough study on HLA-G structure in exudates showed that in exudate fluids HLA-G can form high molecular weight complexes through disulfide bridges, which are present in exudates from patients with inflammatory diseases or cancer. These complexes were produced inside the cells, present in microvesicles (exosomes). In the exosomes, the nature of these HLA-G molecules was not only heterogeneous in size but also in the electrophoretic mobility (16). Human tolerogenic DCs of a new subset were identified that are characterized by their production of IL-10 (17).Called DC-10, these cells are present in vivo in peripheral blood and secondary lymphoid organs, are inducible in vitro from monocytes in the presence of IL-10, and are characterized by the expression of high levels of membrane-bound HLA-G1 and other tolerogenic signaling molecules such as ILT2, ILT3, and ILT4. They are potent inducers of adaptive allo-specific Type 1 regulatory T (Tr1) cells (an inducible subset of Tregs that play a pivotal role in promoting and maintaining tolerance), whose generation critically depends on DC-10 expression of membrane-bound HLA-G1, ILT4, and IL-10 (17, 18).

Other HLA-G immune functions

Other immunomodulatory functions of HLA-G functions have been described. One of them is the capability of sHLA-G to induce apoptosis of NK cells and cytotoxic T cell (CTLs) cells expressing T-cell receptors (TCRs) that can recognize a specific antigen (19), another the capability of soluble and membrane-bound HLA-G to influence the expression and release of IFN-γ by NK cells (20, 21, 22), the capability of soluble and membrane-bound HLA-G to induce the up regulation of inhibitory receptors including its own (23), and the anti-angiogenic function of sHLA-G through CD160 binding (24). By generating a sequence-signal-derived nanopeptide that may bind and
stabilize HLA-E, HLA-G may also increase HLA-E cell-surface expression and so modify NK cells effector function through interaction with activatory or inhibitory CD94/NKG2 complexes (25, 26). Many HLA-G functions were described in vitro, since its expression is critically dependent on micro-environmental parameters. Based on all these findings it is clear the pathological significance of HLA-G expression in vivo in the contexts of cancer (27), transplantation (28), autoimmune diseases, inflammatory diseases, and viral infections (29).

Clinical-translational advances

HLA-G expression in cancer

A high frequency of HLA-G surface expression and increased sHLA-G serum levels have been detected in hematological and solid tumors. HLA-G and sHLA-G expression correlates with a poor clinical outcome in tumor patients. This suggests that HLA-G is an important immune escape mechanism of tumors. HLA-G expression in tumors was first described in melanoma cells (30). Table 1 summarizes HLA-G expression in various tumors and clearly shows that its expression is not an uncommon event in most types of cancer. The frequency of HLA-G expression varies between different types of cancer and even between different studies in the same type of tumor, probably due to the criteria of patient selection and the methodology used. In general, HLA-G expression was found with a higher frequency in choriocarcinoma (31, 32) and the choriocarcinoma cell line JEG-3 is the most widely positive control cell used for studying HLA-G expression (33). When reviewing data in hematological disease HLA-G has not been detected on acute leukemic cells (34-36) with the exception of one study, in which HLA-G was expressed in 18.5% of acute myeloid leukemia (AML) cases (36). HLA-G serum levels were increased in patients with AML, especially in subtypes affecting monocytic and lymphoid lineages as well in patients with B and T acute lymphoid leukemia (ALL). HLA-G protein was also found in cases of cutaneous
lymphoma (8), in chronic lymphatic leukemia (CLL) with significant increase of serum HLA-G (37-41), in classical Hodgkin’s lymphoma (cHL) (42), NHL (43) and in multiple myeloma (MM) (44). In solid tumors expression of HLA-G and detection of sHLA-G is heterogeneous. HLA-G expression is frequent in breast (45–47), endometrial (48, 49) and ovarian cancers (50). It is less frequent in cervical cancer (51). In digestive tumors, HLA-G expression was described in esophageal squamous cell carcinoma (52), colorectal cancer (53), gastric cancer (54) and liver cancer (55). In relation to increased HLA-G expression in cancer, an increase of circulating sHLA-G concentrations was described in patients suffering different types of cancer (56), and the source of this protein can be either the tumoral cells or the reactive immune cells. Data here reported showed an heterogeneity in HLA-G expression in cancer. Intratumour heterogeneity (ITH) as expression of evolutionary dynamics is essential in deciphering the metastatic process in general, as well as eliciting the mechanisms underlying therapeutic resistance. ITH has an effect on the immune response to cancer, since intratumoural mutational diversity can provide neo-antigens that may be perceived by the immune system as non-self, producing unique opportunities for the generation of anti-tumour immunity. On the other hand a discordant mRNA and protein expression in different solid tumors. This reduced frequency of HLA-G-specific protein expression when compared with mRNA transcription could be either because of the post-transcriptional control of the HLA-G expression or related to ITH that may affect modulation of HLA-G expression and immune response within the tumor.

**HLA-G as a diagnostic and prognostic biomarker**

Serum HLA-G antigens are derived from the release of the membrane-bound HLA-G isoforms, such as HLA-G1, and from the secretion of sHLA-G isoforms, such as HLA-G5. sHLA-G is secreted by both immune cells and tumor cells. The monocytes, T cells and DC secrete sHLA-G molecules in vitro. Based on these results, the determination of sHLA-G levels might be used as a diagnostic
tool to distinguish between malignant and benign tumors. HLA-G might serve as a possible marker for tumor sensitivity to chemotherapy and as a prognostic marker for advanced disease stage and clinical outcome. The relationship between stage or prognosis and HLA-G expression in tissue, or variations in its circulating levels, was investigated for several tumors with more frequent HLA-G staining in tumor cells in more advanced stage of the tumor. Some authors have analyzed the role of sHLA-G as circulating tumor marker. The presence of this molecule in biological fluids can derive from the malignant cells, but also from immune cells. As a result, although sHLA-G levels are more elevated in cancer patients than in healthy individuals, further studies are necessary to analyze its discriminant value in relation to benign diseases. sHLA-G plasma levels are significantly elevated in patients with non-small-cell lung cancer (NSCLC) (57). HLA-G assay either in biological fluids and/or in biopsies may have a clinical value in diagnosis, staging or prognosis of cancer, but a prospective validation study should be performed in order to use it as a biomarker. Transplanted patients, who had increased sHLA-G serum levels, had an improved allograft acceptance. These data suggest that increased sHLA-G levels in biological fluids are associated with the down-regulation of immune responses. All data are still heterogeneous in terms of patient selection, and thus are difficult to compare. It is also essential to standardize HLA-G testing, thus, careful testing is necessary in many cases before clinical use can be envisioned.

**HLA-G as a potential therapeutic target**

HLA-G is a potent immune-inhibitory molecule. Given its immune-inhibitory properties, its measurement may help to choose a therapeutic orientation where HLA-G may be targeted. Indeed, it would be important to suppress its immune-suppressive expression in cancer. HLA-G blockade in those tumors that express it remains an attractive therapeutic strategy against cancer. Targeting of HLA-G-expressing cancer cells would be also important for maximize the efficacy of anti-cancer therapies.
Peptides

An experimental approach to target HLA-G expressing cells in a renal cell carcinoma model was the use of HLA-G-derived peptides based on the binding motif to the HLA-A24 (57). HLA-G peptides may induce a cytotoxic attack against HLA-G-expressing HLA-A24 tumor cells suggesting that HLA-G mediated suppression can be overcome using peptide-derived immunotherapy (58).

Effect of systemic treatment on HLA-G expression

Cancer therapies as chemotherapy, radiation therapy, or immunological and biological agents can induce HLA-G production. Increasing dose of gamma-radiation on melanoma cell lines may induce proteolytic cleavage of HLA-G from the plasma membrane, causing a decrease in HLA-G1 cell-surface expression and a concomitant increase in sHLA-G1 concentration in the culture medium (59). The potential of HLA-G induction by the therapy itself could affect the response to treatment and it would be very recommended the measurement of this molecule. Also chemotherapy was shown to induce HLA-G expression, such as 5-aza-2’-deoxycytidine, i.e. decitabine (60). By blocking HLA-G function with a specific antibody, immuno-suppression of the tumor was restored, proving that HLA-G can induce tolerance toward a tumor (16). HLA-G measurement could be of special interest in clinical trials with monoclonal antibodies targeting specific receptors (as trastuzumab or cetuximab) or targeting immunomodulatory checkpoint as Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) or Programmed cell death protein 1 (PD-1). Hypothesis is that increase of sHLA-G may be a predictor of resistance to these monoclonal antibodies.

Conclusions

The immune system not only protects the host against tumor formation but also defines tumor immunogenic phenotype that would develop in immunocompetent hosts. This process called “cancer immunoediting” is divided into three sequential phases in its most complex form (61):
there is a first phase of elimination in which the immune system exerts a surveillance of the tumor development; then a second phase of equilibrium, and a third phase of escape where some tumor cells can evade from immune surveillance. Atopic HLA-G expression can facilitate tumor immune escape by three main mechanisms: (i) cytotoxic attack inhibition, (ii) suppressor T cell induction, and (iii) interference with antigen presentation by DCs. These suppressive functions could be the basis for the elevated association of HLA-G with a higher invasive or metastatic status, or an unfavorable prognosis. Some therapeutics may enhance HLA-G gene expression and contribute to cancer recurrence, its monitoring during cancer therapy can be predictive of response/progression to standard chemotherapy or to immunotherapeutics. Since HLA-G has been proposed as a diagnostic and negative prognostic indicator in cancer, we suggest validating the biomarker in a large prospective case population study, stratifying by stage of disease. Targeting the HLA-G could represent an additional strategy for cancer therapy. Many different molecular inhibitors have demonstrated their ability to selectively inhibit HLA-G expression. These therapeutic agents specific for HLA-G should be tested within phase I, early phase II trials alone or in combination with other therapy protocols for patients with advanced metastatic diseases.

References


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Table 1. HLA-G expression and s-HLA-G in solid tumor biopsies by immunohistochemical analysis.
Figure 1:

Physiological expression → Tolerance

- Placenta
- Villous tree
- Trophoblast cells
- Amniotic fluid

Cytolitic function inhibition

- Throphoblast
- Uterine spiral arteries

Fetal tissues

- Cornea
- Endothelial cells
- Thrombo blasts
- Dendritic cells

- Thymus
- Pancreatic islets
- Macrophages

Adult organs

Membrane HLA-G (HLA-G 1-2-3-4)

Secreted HLA-G (sHLA-G 5-6-7)

- Auto-immune diseases
- Viral infections
- Inflammatory diseases
- Transplantation
- Cancer

Abnormal expression → Immune escape

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Figure 2:

A. Apoptosis induction
- sHLA-G
- Apoptosis
- CD8+
- Cancer cells
- sHLA-G

B. Cytolytic function inhibition
- sHLA-G
- No Ag stimulation
- CD4+
- T-regs
- T cells
- T-reg inhibition

C. T-reg induction
- sHLA-G
- CD8+
- No Ag stimulation
- NK

D. HLA-G Acquisition
- T cells
- NK
- Trogocytosis
- Immune response suppression
- Dendritic cells

HLA-G and s-HLA-G
Solid and hematologic tumors
Diagnostic and/or prognostic markers
Therapeutic targets
Antibodies
Peptides
CD8+
NK

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