Human leukocyte antigen G (HLA-G) is a nonclassical MHC class I molecule that exerts important tolerogenic functions. Its main physiologic expression occurs in the placenta, where it 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 hematologic 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. As HLA-G is a potent immunoinhibitory molecule, its blockade remains an attractive therapeutic strategy against cancer. Elimination of HLA-G-expressing cancer cells would be important in the efficacy of anticancer therapies.
globular domains. HLA-G2 contains the α1 and α3 domains, HLA-G4 contains the α1 and α2 domains, and HLA-G3 is constituted of the α1 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 α3 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 α1 and α3 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, and α3) noncovalently bound to β2-microglobulin (β2-m) and a nanopeptide (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 myelomonocytic 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, for example, differentiation, proliferation, cytolyis, and cytokine secretion. HLA-G antigens can be expressed on tumor-infiltrating immune cells (TIL) as well as in peripheral blood from patients who have cancer (7–9). Due to its tolerogenic effects HLA-G may impair the immune response of patients against tumor. The HLA-G⁺ APC complex can inhibit the function of CD4⁺ T cells and induce their differentiation into regulatory T cells (Treg; ref. 10). Treg, formerly known as suppressor T cells, are a subpopulation of T cells that modulate the immune system, maintain tolerance to self-antigens, and abrogate autoimmune disease (Fig. 1). By indirect mechanisms, HLA-G can exert immune-suppressive activity; specifically it can express the nonclassical 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

Immune inhibition mechanisms of HLA-G receptors can be organized into three groups: (i) the direct immunoinhibitory functions through blocking effector cells; (ii) the indirect immunoinhibitory functions through regulatory cell generation; and (iii) the other functions of HLA-G that have immunoinhibitory consequences.

Inhibition of effector cells

HLA-G is primarily expressed at the maternal–fetal interface by cytotrophoblasts that do not express HLA class I molecules, apart from HLA-C at low levels and HLA-G. In this physiologic context, HLA-G1 is known to inhibit 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 semiallogeneic or allogeneic origin. As shown in Fig. 2A, HLA-G may interact with inhibitory receptors at the surface of the NK cells, leading to their functional inhibition. In addition, it was shown that, through direct interaction with ILT2 or ILT4 inhibitory receptors, HLA-G inhibited CTLs (Fig. 2B).

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 shown 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 2C 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, when generated, HLA–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 (Fig. 2D; ref. 15). 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 interleukin-10 (IL-10; ref. 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 allospecific 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; ref. 19), another the capability of soluble and membrane-bound HLA-G to influence the expression and release of IFN-γ by NK cells (20–22), the capability of soluble and membrane-bound HLA-G to induce the upregulation of inhibitory receptors including its own (23), and the antiangiogenic function of sHLA-G through CD160 binding (3). By generating a sequence-signal-derived nanopetide that may bind and stabilize HLA-E, HLA-G may also increase HLA-E cell-surface expression and, therefore, modify the effector function of NK cells through interaction with activatory or inhibitory CD94/NKG2 complexes (24, 25). Many HLA-G functions were described in vitro, because its expression is critically dependent on microenvironmental parameters. Based on all these findings, it is clear that there is a pathologic significance of HLA-G expression in vivo in the contexts of cancer (26), transplantation (27), autoimmune diseases, inflammatory diseases, and viral infections (28).

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

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>sHLA-G</th>
<th>HLA-G%</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>No</td>
<td>Nr</td>
<td>30</td>
</tr>
<tr>
<td>Choriocarcinoma</td>
<td>No</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Yes</td>
<td>66</td>
<td>46</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>No</td>
<td>40</td>
<td>48</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>No</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Yes</td>
<td>61</td>
<td>50</td>
</tr>
<tr>
<td>Esophageal squamous cell carcinoma</td>
<td>No</td>
<td>90.9</td>
<td>52</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>No</td>
<td>64.6</td>
<td>53</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Yes</td>
<td>49.7</td>
<td>54</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>No</td>
<td>100</td>
<td>55</td>
</tr>
</tbody>
</table>
Clinical–Translational Advances

HLA-G expression in cancer

A high frequency of HLA-G surface expression and increased sHLA-G serum levels has been detected in hematologic 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 (29). 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 (30, 31), and the chorionicarcinoma cell line JEG-3 is the most widely positive control cell used for studying HLA-G expression (32). In reviews of data in hematologic disease, HLA-G has not been detected on acute leukemic cells (33, 34) with the exception of one study, in which HLA-G was expressed in 18.5% of acute myeloid leukemia (AML) cases (35). HLA-G serum levels were increased in patients with AML, especially in subtypes affecting mononcytic and lymphoid lineages as well in patients with B and T acute lymphoid leukemia (ALL). In addition, the HLA-G protein was found in cases of cutaneous lymphoma (8), in chronic lymphatic leukemia (CLL) with significant increase of serum HLA-G (36–40), in classical Hodgkin lymphoma (HL; ref. 41), NHL (42), and in multiple myeloma (43). In solid tumors, expression of HLA-G and detection of sHLA-G are heterogeneous. HLA-G expression is frequent in breast (44–46), endometrial (47, 48), and ovarian cancers (49). It is less frequent in cervical cancer (50). In digestive tumors, HLA-G expression was described in esophageal squamous cell carcinoma (51), colorectal cancer (52), gastric cancer (53), and liver cancer (54). In relation to increased HLA-G expression in cancer, an increase of circulating sHLA-G concentrations was described in patients suffering from different types of cancer (55), and the source of this protein can be either the tumoral cells or the reactive immune cells. Data reported here showed a heterogeneity in HLA-G expression in cancer. Intratumor 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 because intratumoral mutational diversity can provide neo-antigens that may be perceived by the immune system as non-self, producing unique opportunities for the generation of antitumor immunity. On the other hand, it may cause 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 posttranscriptional 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. Monocytes, T cells, and DCs 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 a circulating tumor marker. The presence of this molecule in biologic fluids can derive not only from the malignant cells, but also from immune cells. As a result, although sHLA-G levels are more elevated in patients with cancer than in healthy individuals, further studies are necessary to analyze its discriminatory value in relation to benign diseases. sHLA-G plasma levels are significantly elevated in patients with non–small cell lung cancer (56). HLA-G assay, either in biologic fluids or in biopsies, may have a clinical value in diagnosis, staging, or prognosis of cancer, but a prospective validation study should be conducted 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 biologic fluids are associated with the downregulation 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 in the choice of 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 might serve as a possible marker for tumor sensitivity to chemotherapy and as a prognostic marker for advanced disease stage and clinical outcome. Targeting of HLA-G–expressing cancer cells would be also important for maximize the efficacy of anticancer 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 (56). 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 (57).
Effect of systemic treatment on HLA-G expression

Cancer therapies such as chemotherapy, radiation therapy, or immunologic and biologic agents can induce HLA-G production. Increasing dose of γ-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 (58). The potential of HLA-G induction by the therapy itself could affect the response to treatment, and measurement of this molecule is recommended. In addition, chemotherapeutic agents have been shown to induce HLA-G expression, such as 5-aza-2'-deoxycytidine, i.e., decitabine (59). By blocking HLA-G function with a specific antibody, immunosuppression of the tumor was restored, proving that HLA-G can induce tolerance toward a tumor (60). HLA-G measurement could be of special interest in clinical trials with monoclonal antibodies targeting specific receptors (e.g., trastuzumab or cetuximab) or targeting immunomodulatory checkpoint as cytokotoxic T-lymphocyte antigen 4 (CTLA-4) or programmed cell death protein 1 (PD-1). The 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 (60): 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, and its monitoring during cancer therapy can be predictive of response/progression to standard chemotherapy or to immunotherapeutics. As 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 HLA-G could represent an additional strategy for cancer therapy. Many different molecular inhibitors have shown their ability to selectively inhibit HLA-G expression. These therapeutic agents specific for HLA-G should be tested within phase I and early phase II trials alone or in combination with other therapy protocols for patients with advanced metastatic diseases.

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Development of methodology: C. Curigliano, C. Criscitiello
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G. Curigliano
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G. Curigliano
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): G. Curigliano
Study supervision: G. Curigliano, C. Criscitiello
Other (final editing of the figure): G. Curigliano

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