HLA-G: Facts and Fictions

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Abstract

Human leukocyte antigen (HLA)-G is a nonclassical MHC class I molecule with modulatory effects on NK and T cells. Unlike classical HLA class I molecules, HLA-G has seven isoforms, three of which are soluble. Soluble HLA-G molecules are reportedly able to transduce negative signals to immune cells after interacting with their corresponding receptors. The expression of these molecules plays significant roles in maternal tolerance against semi-allogenic fetuses. Overexpression of HLA-G in tumors and increased serum levels of soluble HLA-G have been reported in different malignancies, and these changes may be involved in tumoral immune evasion and cancer progression. To improve immune responses against tumor cells, the downmodulation of HLA-G by siRNA or blocking monoclonal antibodies can be helpful in cancer immunotherapy. Additionally, HLA-G can be considered a potential biomarker for the diagnosis and/or prognosis of certain cancers. Although polymorphism of the HLA-G gene-coding region is more limited than in classical HLA class I, some genetic variations in regulatory regions of the gene control the expression level of this molecule. Furthermore, epigenetic factors such as infections may affect the expression of HLA-G in infection-related cancers.

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HLA-G: from gene to protein

HLA-G is a nonclassical class I MHC molecule encoded by a gene on chromosome 6, downstream from the classical HLA class I genes. It contains eight exons which encode signal peptide, α1, α2 and α3 domains, and the transmembrane and cytoplasmic region. Due to the presence of a stop codon in exon 6, the last two exons are not translated. To date, the 58 alleles reported for this gene are believed to encode only 18 different proteins (http://hla.alleles.org/nomenclature/stats.html), because most exonic variations are synonymous and the remaining variation sites are intronic or located in regulatory regions [1].

HLA-G generates seven different isoforms through alternative splicing. Four of them are membrane-bound (G1-G4) and three isoforms are soluble forms (G5-G7). HLA-G1 contains a complete α chain with three domains and is expressed in combination with β2-microglobulin. HLA-G2 contains the α1 and α3 domains, HLA-G3 contains the α1 and α2 domains, and HLA-G4 consists of a single α1 domain. HLA-G5, -G6 and -G7 are soluble analogues of HLA-G1, -G2 and -G4, respectively (Figure 1) [2]. Due to the existence of intron-4 or -2 in the mRNA of soluble isoforms which contain the stop codon, the transmembrane and cytoplasmic domains of HLA-G5, -G6 and -G7 are not translated [1]. Soluble HLA-G (sHLA-G) can also be generated through proteolytic cleavage of membrane-bound isoforms. HLA-G can also form dimers via disulfide bonds through Cys-42 in the α1 domain, which enhance the avidity of the molecule for its corresponding receptors [2].

HLA-G receptors

HLA-G interacts with immune cells through multiple receptors such as immunoglobulin-like transcript (ILT)2, ILT4, and killer-cell immunoglobulin-like receptor (KIR2DL4). ILT2 is expressed by natural killer (NK) cells, T cells, dendritic cells (DCs) and decidual macrophages. Expression of ILT4 is limited to myeloid cells and DCs, whereas KIR2DL4 is expressed in all NK cells [3]. Although these receptors can bind both classical and nonclassical HLA class I molecules, they have higher affinity for HLA-G than classical HLA class I molecules. Moreover, in contrast to classical HLA class I, the interaction of HLA-G molecules on tumor cells with ILT2 on NK cells does not require tumor cell lipid raft integrity [4].

ILT2 and ILT4 possess four and three immune
receptor tyrosine-based inhibitory motifs (ITIMs), respectively, in their long cytoplasmic tails. These receptors contain four extracellular domains, D1–D4, and mediate the interaction of these receptors with HLA class I molecules. However, KIR2DL4 has a long cytoplasmic tail, like inhibitory KIRs, and also has a charged amino acid (arginine) in the transmembrane domain which can act to activate KIRs. It has been shown that because of conformational prevention, KIR2DL4 cannot bind HLA-G dimers [3].

Another recognized HLA-G receptor is CD160, expressed by endothelial cells. Engagement of the CD160 receptor by HLA-G triggers the apoptosis of endothelial cells and inhibits their proliferation and the angiogenic process [5].

**HLA-G immune function**

HLA-G exerts immunomodulatory functions via direct and indirect mechanisms. HLA-G interacts with inhibitory receptors on NK cells and cytotoxic T lymphocytes (CTLs), leading to their functional inhibition [6]. It has been shown that sHLA-G is able to induce apoptosis in CTLs [7]. HLA-G induces upregulation of inhibitory receptors, including itself [8], and also has indirect inhibitory functions through the generation of regulatory cells. HLA-G-induced regulatory cells require HLA-G for their generation but not for their function [9]. For example, HLA-G1-transfected antigen presenting cells (APCs) lead to CD4+ and CD8+ T cell unresponsiveness to alloantigens, and allow them to acquire a regulatory phenotype [10]. Another indirect immunomodulatory effect of HLA-G arises from HLA-G and HLA-E cooperation. HLA-E directly binds to HLA-G-derived signal peptide, and then interacts with CD94/NKG2A, the inhibitory receptor on NK cells [11].

**The HLA-G story**

HLA-G was discovered by Geraghty et al. in 1987 [12], and its modulatory functions in the trophoblast during pregnancy were initially described in 1990 [13]. Semi-allogeneic fetuses evade rejection by maternal leukocytes via different mechanisms such as expressing Fas ligand (FasL), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), indoleamine 2,3-dioxygenase (IDO) and programmed death-ligand 1 (PD-L1). In addition to these immunomodulatory molecules, the extravillous trophoblast (EVT) which invades the maternal uterine mucosa lacks HLA-A and HLA-B molecules, but expresses HLA-C, -E and -G [14-15]. HLA-G was first introduced as a key molecule for the maintenance of maternal tolerance by NK cell inhibition [16]. The trophoblast is recognizable by NK cells thanks to the absence of HLA class I molecules, but HLA-G expression on the trophoblast helps this tissue to avoid NK cell-mediated lysis [15]. The immunosuppressive effects of HLA-G were supported by the detection of decidual DC-10, a major APC subset in decidua, which expresses high levels of HLA-G which in turn induce ILT4 on infiltrating immune cells and maintain tolerance during pregnancy [17].

In addition to protecting the fetus from maternal immune cell attack, HLA-G expression on EVT plays an important role in uterine vascular remodeling. Unlike peripheral NK cells, decidual NK cells (dNK) are less cytotoxic and mostly secrete cytokines and growth factors such as IFN-γ, TGF-β, VEGF, placental growth factor (PLGF) and angiopoietin 1/2 [18]. Moreover, β2m-bound HLA-G isoforms are not expressed on distal EVT, and these invasive cells express only heavy chain-containing HLA-G isoforms which may have limited inhibitory effect because they are not recognized by ILT2 [19-20]. Decidual NK cells, which express more KIR2DL4 than ILT2, secret IL-6, IL-8 and TNF-α after interaction with HLA-G, and this subsequently leads to NK cell-mediated EVT invasion into the decidua [21]. Therefore, HLA-G can play potentially important roles in maternal tolerance of the fetus through different mechanisms.

Aberrant HLA-G expression has been observed under nonphysiological conditions such as viral infection, cancer, transplantation, and in inflammatory and autoimmune diseases. HLA-G may have two distinct effects in pathological conditions: it can be useful in inflammatory and autoimmune diseases, or dangerous in tumors and infections [3]. In many studies, increased HLA-G expression was shown to be associated with graft acceptance and the prevention of chronic rejection [22].

**HLA-G and cancer**

Malignant cells typically develop mechanisms to evade immune surveillance [23]. HLA class I downregulation and HLA-G overexpression play critical roles in the modulation of immune responses against tumors. Because tumor cells express mutated or normal but downregulated antigens, they can be recognized as missing-self, non-self and stress-induced self by the immune system. This is the first step in the immune editing mechanism called the elimination phase. In this primary phase, most tumor cells are recognized by the immune system and eliminated by NK cells and CTLs. When a tumor is not eradicated completely, resistant variants may be selected: this mechanism selects cells that are less immunogenic, and gives rise to the equilibrium phase. When immune-resistant variants are maintained and expanded, the evasion phase begins [24-25]. HLA-G molecules play crucial roles in all phases of immune editing by inhibiting the cytolytic action of NK cells and CTLs [7-26]. HLA-G-positive tumors might progress from the elimination phase to the equilibrium phase through NK cell inhibition [26-27]. These HLA-G-positive cells are less immunogenic and more resistant to immune system recognition and cytolysis. Therefore they are positively selected and lead to the evasion phase, characterized by rapid tumor growth [24].

HLA-G was shown to be expressed by melanoma cells in 1998 [28]. In the following years, HLA-G expression was frequently detected in different solid tumors [29-31]. What emerged from these studies was that HLA-G could potentially be expressed by all tumor types. Although HLA-G is mostly expressed by tumor cells rather than the surrounding healthy tissues [32], expression levels
were reported to be highly variable (from 0% to 100%) in different tumors. Moreover, plasma levels of sHLA-G are significantly higher in patients with different malignancies than in healthy controls [33-35].

Many recent studies have assessed the clinical relevance of HLA-G expression in different cancers, as summarized in Table 1. In general, high HLA-G expression correlates with advanced disease stages, poor histological grade, metastases, shorter survival times and tumor recurrence [29-30, 40, 43]. It was also previously demonstrated that HLA-G overexpression in solid tumors correlates positively with the number of infiltrating Treg cells, and negatively with CD8-positive T cell and NK cell activities [44-45]. No association has been reported between increased HLA-G expression and clinicopathological aspects in malignancies such as bladder cancer [46] and acute myeloid leukemia [47]. However, we recently found a correlation between HLA-G overexpression in tumor tissues and advanced tumor stages and grades in pancreatic adenocarcinoma, although we found no correlation between HLA-G expression and clinicopathological parameters in hepatocellular carcinoma (HCC) [48].

HLA-G displays different faces in hematological malignancies. In many studies, increased HLA-G expression was shown to be associated with a worse prognosis [49-50], although high plasma levels of sHLA-G showed no correlation with prognosis [51-52]. In contrast, several studies found evidence of a relationship between HLA-G and better prognosis for B cell neoplasms [53-54]. These different observations can be attributed to the capacity of B cells to express ILT2 and therefore inhibit the proliferation of malignant B cells [55]. The fate of HLA-G interaction with its receptor on malignant B cells is determined by the balance between HLA-G-driven inhibition on these cells (as immune effector cells) and the antiproliferative effect on these cells (as target malignant cells) [55]. Heterogeneous patterns of HLA-G expression in different tumors may reflect differences in the biology of different types of tumor, genetic diversity among the populations studied to date, and the sensitivity of methods used to detect HLA-G [56]. In addition, the tumor microenvironment and viral infections may also have some impact on HLA-G expression [57], as discussed later in this review.

Associations between increased sHLA-G plasma levels and poor prognosis for tumoral diseases have been reported in patients with HCC, lung cancer and colorectal cancer [2-34, 35]. However, no clear association was observed between sHLA-G plasma levels and clinicopathological features in breast cancer or gastric cancer [38-58].

The evaluation of HLA-G might be used as a diagnostic tool to distinguish between malignant and benign tumors [31]. In addition, HLA-G might serve as a prognostic marker for the clinical outcome [36-41]. HLA-G may also be a potential target for cancer immunotherapy in combination with other therapeutic strategies.

HLA-G promotes cancer development by different mechanisms. This molecule is involved in tumor immune escape not only by inhibiting NK cells but also by inducing apoptosis in NK cells and CTLs [27-59]. Moreover, in many tumors HLA-G expression was reported to be positively associated with local Treg numbers [44-45]. Soluble HLA-G, on the other hand, is able to downregulate chemokine receptors on lymphocytes and inhibit the chemotactic migration of NK, T and B cells into the tumor site [60-62]. As previously noted, HLA-G and HLA-E might cooperate in immune inhibition. Zeestaten et al. have shown that the absence of HLA-E and HLA-G on colon tumor cell surfaces was related to longer overall and disease-free survival [63]. Another study of colorectal cancer found that single or double expression of HLA-G and HLA-E was associated with shorter overall survival. In addition, HLA-E expression correlated significantly with tumor metastases. Another study reported that HLA-G expression alone can be used as a prognostic marker for overall survival [64]. HLA-G may also be involved in aggressive behavior of tumors and metastasis through the upregulation of matrix metalloproteinase 15 [65-66]. Another mechanism called trogocytosis, i.e. the transfer of partial membranes with associated molecules via cell-cell contact [67], sometimes occurs between HLA-G-expressing tumor cells and NK or T cells, which makes the latter act as suppressor cells [68-70].

It was recently demonstrated that tumor cells secrete HLA-G-bearing extracellular vesicles. Riteau et al. first explained the presence of HLA-G-bearing extracellular vesicles in the supernatant of an HLA-G-positive melanoma cell line [71]. In this connection, König et al. and Grange et al. showed that these vesicles could modulate immune cell functions in kidney and breast cancers in ways that lead to cancer progression [38-72].

HLA-G expression can be affected after cancer progression. Genetic instability and epigenetic changes due to progression may contribute to the development of less immunogenic tumors with increased HLA-G and decreased classical HLA class I molecules [56]. Furthermore, chronic inflammation and hypoxia in advanced tumors seem to induce HLA-G expression [73-74]. HLA-G also may play a critical role in the creation of appropriate conditions for tumor progression, by generating tolerogenic APCs and Treg cells [75]. Upregulation of HLA-G might be induced by these regulatory/tolerogenic cells, which produce IL-10 and TGF-β in the tumor microenvironment. This positive loop usually occurs in patients with advanced-stage cancer [76-77].

**HLA-G as a target in cancer therapy**

Targeting the HLA-G molecule is one of several promising approaches for cancer therapy. In 2011, Agaúgué et al. reported improved tumor lysis with a blocking monoclonal antibody against HLA-G in C57BL/6 mice which had been inoculated with the M8-HLA-G1 cell line [78]. The effect of blocking antibodies against HLA-G was also investigated by Maki et al., who observed that HLA-G blockade increased the susceptibility of chronic lymphocytic leukemia to NK cell-mediated killing [79]. Favier et al. also demonstrated that blocking ILT2 or HLA-G with a monoclonal antibody
restored NK cell cytolytic activity on LCL 721.221 cells (a B lymphoblastoid cell line) which expressed HLA-G through transfection with HLA-G1 cDNA [80]. In another study, bone marrow-derived mesenchymal stem cells (MSCs) were treated with IL-10 to increase the secretion of HLA-G5. Neutralization of sHLA-G with an antibody suppressed the formation of CD4+CD25 high/Foxp3+ Treg cells and facilitated allogeneic T cell proliferation [81].

Zeng et al. showed that using HLA-G siRNA to decrease HLA-G expression on a human HCC cell line led to increased NK cell-induced tumor cytolysis [82]. This was despite the fact that HLA-G/ILT2 interaction can suppress the proliferation of Raji cells, a B cell line in Burkitt’s lymphoma. Therefore, in contrast to solid tumors, the administration of an HLA-G blocking antibody or HLA-G siRNA may lead to enhanced proliferation of malignant lymphocytes [54]. Our group recently reported improved NK cell cytotoxicity against SKOV3 cells after HLA-G downregulation with siRNA [83].

**HLA-G and infection-related cancers**

In infectious diseases, HLA-G has been shown to have deleterious effects by promoting pathogen escape from immune recognition [84]. Although it was found that oncoviruses directly induce cancer, some nononcogenic pathogens may also lead to malignancy by inducing chronic inflammation [85]. The mechanism of tumorigenesis by infectious agents has not been fully elucidated; however, it is clear that both host and pathogen are involved in this process [86]. In this regard, the main question is whether HLA-G plays role in tumorigenesis following infections.

Helicobacter pylori has been known as a dominant species living in the human stomach, where colonization by this pathogen sometimes leads to chronic inflammation, called gastritis. Although H. pylori is considered a strong risk factor for gastric cancers, only a small number of infected individuals develop malignancy. The risk of malignancy is highly related to both bacterial strain and host responses [87]. The only study to explore the relationship between HLA-G and H. pylori was that by Souza et al., who observed HLA-G expression in gastric tissues from 79.6% of patients with H. pylori infection. They found a negative association between the presence of HLA-G and colonization by H. pylori; however, the role of HLA-G in the induction of gastric cancer was not clarified.
In this study [88]. Recently, our group explored HLA-G expression in patients with H. pylori infection and gastric cancer. Our results showed that 32% of gastric cancer samples were positive for H. pylori, whereas only 12% of the samples were positive for both H. pylori and HLA-G (unpublished data).

Hepatitis B virus (HBV) is a known risk factor for HCC [86]. Park et al. detected higher levels of sHLA-G in patients with active HBV and HCC than healthy controls. Their results also showed increased levels of sHLA-G in early stages of HBV-mediated HCC [89]. However, in a recent study, we observed no correlation between HLA-G expression and the presence of HBV genome in HCC tissues [48]. Dong et al. observed a trend toward increasing HLA-G expression with tumor progression in patients with cervical intraepithelial neoplasia. They also detected higher expression of HLA-G in human papillomavirus (HPV)-positive patients than HPV-negative patients with this disease [90]. Guimarães et al. reported low HLA-G5 levels in their patients with HPV-related cervical cancers. They also observed lower HLA-G5 levels in invasive cervical cancers without metastases compared to patients with metastases [91]. In recent work by our group on gynecologic cancers, higher levels of HLA-G expression were detected in cervical cancer than other cervical cancers. They also observed lower HLA-G5 levels in their patients with HPV-related papillomavirus (HPV)-positive patients than HPV-negative patients with this disease [90]. Guimarães et al. reported low HLA-G5 levels in their patients with HPV-related cervical cancers. They also observed lower HLA-G5 levels in invasive cervical cancers without metastases compared to patients with metastases [91]. In recent work by our group on gynecologic cancers, higher levels of HLA-G expression were detected in cervical cancer than other gynecologic cancers, and HPV-DNA was detected in 65% of these lesions (unpublished data).

HLA-G expression may be controlled more directly by local microenvironmental factors than by infectious agents. This hypothesis was supported by Gazit et al., who observed HLA-G induction by culture conditions such as nutrient deficiency, hypoxia, or both in an EBV-transformed B cell line [92].

**HLA-G: regulation of gene expression**

In addition to epigenetic factors, HLA-G expression is controlled by one or more inherited alleles and genetic variations in noncoding regions of the gene [93-94]. Therefore, the expression level of HLA-G after infections appears to be determined, at least in part, by the host’s genetic background.

Low expression of HLA-G due to promoter hypermethylation was reported to be associated with preeclampsia [95]. Although HLA-G overexpression has been documented in several cancers, the role of HLA-G promoter demethylation in these processes has not been clarified. Gillio-Tos et al. found no relation between HLA-G promoter demethylation and HPV-induced cervical intraepithelial neoplasia [96].

It was believed that HLA-G expression might also be affected by polymorphisms in the 5' upstream regulatory region (5'UTR), because transcription factor binding sites are mostly located in this region, e.g., cyclic AMP-dependent transcription factor-1 (ATF-1), Ras responsive element-binding protein1 (RREB1), interferon regulatory factor-1 (IRF-1) and nuclear factor (NF)-κB [97].

HLA-G expression is known to be induced by local changes resembling hypoxia, or by the presence of progesterone. Hypoxia response element is located in the upstream region of the HLA-G gene, at positions −242 to −238 [98]. The progesterone receptor, after binding to its ligand, is also bound to progesterone response element in the HLA-G promoter at positions −52 and −38 [99]. It has also been reported that HLA-G expression can be upregulated by IL-10, cortisol and some drugs such as methotrexate, albeit via unknown pathways [77, 100, 101].

In addition to factors involved at the transcriptional level, HLA-G expression is also controlled at post-transcriptional levels [102]. Although some microRNAs bind to nonpolymorphic sequences, polymorphisms in the HLA-G 3' untranslated region (3' UTR) may affect mRNA stability by changing the affinity of this region for the corresponding microRNAs [94, 102]. Moreover, HLA-G expression is also affected by the level of related microRNAs. Bian et al. have shown that decreased miR-152 expression leads to HLA-G upregulation and impaired NK cell-mediated antitumor response [103]. Jasinski-Bergner et al. also reported that miR-548q and miR-628-5p influenced HLA-G downregulation and enhanced NK cell-mediated cytolysis [104]. One of the most frequently studied polymorphisms in 3' UTR is the 14-bp insertion/deletion (ins/del). A link between the 14-bp del/del genotype and higher levels of HLA-G production was reported in healthy people [105].

A meta-analysis by Zhang et al. explored the role of HLA-G 14-bp ins/del polymorphism in the susceptibility to cervical cancer, HCC, esophageal cancer, neuroblastoma and papillary thyroid carcinoma. They found a prominent role for this polymorphism in susceptibility to HCC but not to the other cancers they reviewed [106]. However, the association between this polymorphism and cancer susceptibility may differ among populations [97]. For example, the frequency of the HLA-G 14-bp del/del genotype was higher in patients from southeastern Iran who had breast cancer compared to the control population [107], whereas this genotype showed no association with breast cancer in patients from northwestern Iran [108]. Therefore, HLA-G polymorphisms might be predictive markers for susceptibility to some types of cancer. However, additional studies of populations representing different ethnicities and with larger sample sizes are required to confirm this hypothesis.
In conclusion the extensive immunomodulatory effects of HLA-G on malignancies have opened a new window for the diagnosis, prognosis and treatment of cancers. HLA-G is a potential new biomarker for the differential diagnosis between malignant and benign tumors. At this time, however, HLA-G is a potential target molecule in therapeutic approaches to enhance the efficacy of cancer treatments. Therefore, more in vitro and in vivo studies are needed to determine the therapeutic potential HLA-G molecules.

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