HLA-G and Immune Evasion in Cancer Cells

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Acquisition of novel gene products or new antigens in cancer cells elicits a host immune response that results in selection pressure for tumor clones to evade immunosurveillance. Similar to maternal–fetal tolerance and allotransplantation acceptance, upregulation of HLA-G expression has been found as one of the mechanisms that are programmed in cancer cells. HLA-G expression is frequently detected in a wide variety of human cancers and its protein levels negatively correlate with poor clinical outcome. The immune inhibitory effect can be achieved by binding of HLA-G molecules to the immunoglobulin-like inhibitory receptors that are expressed on the immunocompetent cells at all stages of the immune response. This review summarizes recent studies of HLA-G expression in human cancer, with a special focus on the molecular mechanisms that underlie how HLA-G molecules facilitate tumor cell evasion of the host immune response, and presents new directions for developing HLA-G-based diagnosis/therapeutics.

Key Words: cancer, HLA-G, immunosurveillance, therapeutics

In normal human tissues, constitutive HLA-G expression is restricted to a few tissue types, including trophoblastic cells and thymic epithelium. The identification of HLA-G expression in trophoblasts and its role in suppressing local immunity in the placenta suggest that cancer cells employ HLA-G overexpression during tumor development to help evade host immunosurveillance; a strategy that is similar to that of trophoblastic at the maternal–fetal interface. Indeed, HLA-G expression has been detected in a wide variety of human cancers, including cutaneous melanoma, lung carcinoma, ovarian carcinoma, endometrial carcinoma, gastric carcinoma, hematopoietic tumors, renal cell carcinoma, mesothelioma, breast carcinoma, trophoblastic tumors, glioma, bladder carcinoma, and colorectal carcinoma, and lymphoproliferative disorders. In addition, the soluble form of HLA-G (sHLA-G) can also be detected in the supernatant of body fluids or malignant effusions in cancer patients. These studies have important biological and clinical implications for HLA-G expression in human tumor tissues. The above tumor types might only
represent a short list and more human cancers with HLA-G expression will be reported as research progresses.

This review summarizes those recent studies of HLA-G expression in human cancer, with a special emphasis on the molecular mechanisms of how HLA-G molecules inactivate immune effectors. Understanding the HLA-G-regulated immune response is fundamental to elucidation of the molecular mechanisms in cancer development and lays the foundation for future therapeutics by targeting HLA-G and related molecules.

**HLA-G is a Distinct Major Histocompatibility Complex (MHC) Class I Molecule**

HLA-G is a unique, non-classical (class Ib) MHC class I molecule and, like other class I MHC proteins, it is composed of a membrane-bound heavy chain and a nonameric peptide that associate with each other via a non-covalent protein–protein interaction. The heavy chain of HLA-G requires association with the β2m molecule for its exportation to the cell surface and for binding to HLA-G ligands.1,17 Although the HLA-G gene is located in the HLA-1 locus of human chromosome 6 and shares several similar characteristics with other MHC class I molecules, its expression pattern, peptide binding properties, and immunological functions are different. More importantly, the expression levels of classical MHC molecules (class Ia), HLA-A, HLA-B and HLA-C, are usually downregulated in tumor cells. This indicates different patterns of expression of the various classes of MHC molecules in the development of human cancer, which might act in concert for the tumor-associated immunosuppressive phenotype.18,19

Protein structure analyses demonstrate that the membrane bound heavy chain of HLA-G contains three immunoglobulin-like domains (α1–α3) in which the α1 and α2 domains constitute the peptide-binding cleft. For the classical (class Ia) MHC molecules, the peptide-binding regions are characterized by extensive amino acid polymorphism, which creates a diverse repertoire for peptide-loading and T-cell recognition.20 In contrast, non-classical HLA molecules such as HLA-G contain limited polymorphism, which results in a restricted number of peptides that are capable of binding to HLA-G.21,22 To date, only three peptides have been isolated and characterized from placenta-derived HLA-G molecules,23 and other HLA-G binding peptides remain to be identified from human cancer cells.

In addition, unlike classical MHC class I molecules, HLA-G appears inefficient at presenting exogenous peptides because it does not contain most of the intracellular portion that other MHC class I proteins do.24 The structural uniqueness of HLA-G is therefore translated to its distinct biological features in cellular functions. For example, HLA-G functions as a common ligand for inhibitory receptors on immune effectors, thus participating in immune regulation. It has also been shown that the identity of the peptide on the peptide-binding cleft affects the binding of HLA-G to killer cell immunoglobulin-like receptor (KIR).25 Another feature unique to HLA-G which is not shared by other MHC molecules is its homodimerization to form the cys42–cys42 disulfide-linked complexes.26,27 Dimerized HLA-G proteins, in contrast to the monomeric form, have a higher protein stability and confer a higher affinity to the inhibitory receptors, which in turn can contribute to more potent immunosuppression in immune effector cells.17,26–28

**Clinical Evidence of HLA-G in Immunosuppression**

Clinical evidence in support of the role of HLA-G in immunosuppression primarily comes from studies that have focused on correlating HLA-G expression levels and clinical outcome in pregnancy and organ transplantation, which represent two major conditions for a host response to non-self tissues. In pregnancy, it is accepted that maternal immunotolerance to the “semi-allograft”,
that is, fetus and placenta, is attributed to the presence of HLA-G. The fact that trophoblast cells in human placenta express a very high level of HLA-G suggests its involvement in regulating immune reactions at the fetal–maternal interface. Secretion of sHLA-G by the early conceptus appears to be essential for successful implantation, and it has been used as a reliable marker of increased subsequent pregnancy following in vitro fertilization. The presence of HLA-G transcripts in preimplantation embryos is correlated with increased blastocystic cleavage rate and a greater number of blastomeres per embryo following in vitro fertilization. In contrast, the abortion rate increases for women who have previously received embryos with low or undetectable HLA-G expression. A null HLA-G allele in a conceptus is also associated with recurrent miscarriage in women.

Allogeneic organ transplantation is always rejected by the host immune surveillance system unless immunosuppressive reagents are administered to protect the grafted tissues. sHLA-G is usually detected at high levels in blood samples from transplantation patients whose grafted tissues survive. In addition, HLA-G-positive infiltrating monocytes are observed in the grafted tissues due to the binding of sHLA-G to its receptor on the immune cells. The finding of the above clinical correlation studies are consistent with the view that HLA-G expression plays an important role in immunosuppressive functions in pregnancy and allograft rejection. It is very likely that similar molecular mechanisms also are used by cancer cells to evade host immunosurveillance.

**Variant Forms of HLA-G in Human Cancer**

In addition to the membrane-bound form of HLA-G, increased plasma levels of sHLA-G have been detected in patients with malignant tumors. These findings suggest that HLA-G participates in the immune response and networking via direct cell–cell contact and through secretion of sHLA-G (Figure 1). Clinical studies on tumor specimens have demonstrated that monomeric and dimeric sHLA-G can be detected in body fluids, including serum and effusion samples, and sHLA-G shares similar biological functions with the membrane-bound form. These findings could explain why only a small percentage of tumor cells show positive HLA-G staining in human tumor tissues. By secreting sHLA-G that diffuses into deeper tissues, tumor cells still can inactivate the local immune response and networking.
response, even for those bystanders without HLA-G expression on the cell surface.

Production of sHLA-G can be achieved by mRNA splicing. In fact, seven different HLA-G isoforms have been reported and they are found in a tissue-specific expression pattern. These isoforms include four membrane-bound HLA-Gs (HLA-G1 to G4) and three sHLA-Gs (HLA-G5 to G7). HLA-G1 represents the full-length version of HLA-G and contains a signal peptide (exon 1); the α1, α2 and α3 domains (exons 2–4, respectively); the transmembrane domain (exon 5); and a short intracellular domain (exons 6 and 7). HLA-G2, as compared with HLA-G1, does not contain exon 3, and HLA-G3 does not contain exons 3 and 4, whereas HLA-G4 does not contain exon 4. Isoforms HLA-G5 to G7 retain a portion of intron 4 that contains a stop codon, which results in expression of truncated (without exon 5 transmembrane domain) or soluble forms that correspond to HLA-G1 to G3, respectively.

Factors Involved in Regulation of HLA-G Expression in Tumors

The molecular mechanisms that upregulate HLA-G expression in tumor cells are complex. They involve several factors including epigenetic control of the HLA-G promoter activation and a variety of environmental factors such as hypoxia, stress, hormones, certain cytokines, and viral infection. Sequence analysis of the HLA-G gene promoter reveals that almost all regulatory boxes described for classical MHC class I genes are not conserved in the HLA-G promoter. This finding suggests that the mechanisms involved in the transcriptional regulation of HLA-G expression are unique and partly independent of those that regulate classical HLA-class I genes.

Recent studies have demonstrated that HLA-G gene transcription activity can be controlled by cis-acting epigenetic mechanisms that include DNA methylation and histone acetylation. For example, primary tumor cells in culture gradually decrease HLA-G expression, and cells treated with histone deacetylase inhibitors or DNA demethylating agents re-express HLA-G by reversing the promoter silencing. The epigenetic regulation is even more pronounced under certain microenvironmental conditions, such as hypoxia, which is a common phenomenon in solid tumor tissues. Stabilization/activation of hypoxia-inducible factor I is the key cellular response under hypoxic conditions. Hypoxia-inducible factor I acts as a transcription regulator that controls expression of a wide variety of genes, including HLA-G in response to hypoxia. Gazit et al have recently demonstrated that hypoxia upregulates HLA-G in Epstein–Barr-virus-transformed B-cell lines but not in freshly isolated peripheral blood lymphocytes, which suggests that oncogenic viruses also play a role in hypoxia-induced HLA-G upregulation.

A tumor microenvironment is enriched by a variety of cytokines that are released by tumor-infiltrating lymphocytes. The interaction of cytokines and tumor cells can regulate HLA-G expression. For example, granulocyte–macrophage colony-stimulating factor and interferon (IFN)-γ that are secreted by infiltrating cytotoxic T cells have been shown to enhance HLA-G expression in tumor cells. Secretion of interleukin (IL)-10 by tumor cells can also upregulate HLA-G expression in tumor tissues through an autocrine or paracrine mechanism. HLA-G, in turn, modulates cytokine expression in immune cells to produce a profile of Th2-type cytokines, including IL-10, IL-4 and IL-3. Clinical studies have shown that this shift towards the Th2 cytokine profile, especially the enhanced IL-10 secretion, is frequently associated with impairment of antitumor immunity.

Molecular Mechanisms of HLA-G in Immunosuppression

Tumor development and progression are always accompanied by expression of novel tumor-associated antigens that can elicit an immune reaction by activating immune defenders such as
cytotoxic T cells, natural killer (NK) cells, and macrophages. In recent decades, great efforts have been made to develop immunotherapy against cancer by applying tumor-specific cytotoxic T-cells to cancer patients. Although this T-cell-based approach has shown some promise in preclinical models and clinical trials, only moderate success has been achieved to date. Tumor clones equipped with the ability to evade immune recognition and destruction undergo clonal expansion, which ultimately leads to tumor recurrence that is refractory to the previous immunotherapy.

One of the possible mechanisms that cancer cells employ to overcome vigilant immunosurveillance and hostile attack involves expression of HLA-G. A growing body of evidence has demonstrated that direct interaction between HLA-G and leukocyte immunoglobulin-like receptors, LILRB1 (also known as LIR1/ILT2/CD85J) and LILRB2 (LIR2/ILT4/CD85D), and between HLA-G and KIR2DL4 (CD158D), belong to a family of immunoreceptors that are expressed on T cells, B cells, monocytes (macrophages), myeloid dendritic cells (DCs), and NK cells, which upon ligand binding can inactivate those immune effectors. On the other hand, KIR2DL4 belongs to the gene family of killer cell inhibitory receptors, which upon binding to HLA-G inhibit NK-cell-mediated cytolytic activity. Although these immunoglobulin-like receptors also interact with other HLA class I ligands, they show the highest binding affinity to HLA-G. HLA-G-binding ligands share a common cytoplasmic immunoreceptor tyrosine-based inhibitory motif sequence, which upon HLA-G binding can recruit intracellular protein-tyrosine phosphatases and trigger an inhibitory signal cascade. The negative signaling, in turn, downregulates the activation of various immunoresponsive genes and affects the cytokine/chemokine profiles secreted by the effector cells. As a result, cell maturation and clonal expansion of immunocompetent cells are significantly reduced. Membrane-bound and secreted forms of HLA-G are capable of upregulating the expression levels of its binding ligands, the immunoglobulin-like inhibitory receptors. Therefore, these effector cells become more sensitive to the HLA-G-mediated inhibitory effects, which results in immune tolerance of tumor cells.

Tumor immunology consists of complex dialog between cancer cells and a variety of resident immune effectors within the tumor microenvironment. In the following sections, we briefly summarize the receptors that are known to interact with HLA-G, and the biological effects of HLA-G on immune cells, including T-lymphocytes, NK cells, DCs, and B-lymphocytes (Figure 2).

**Interactions with T lymphocytes**

In cell-mediated immunity, the interaction between antigen-presenting cells and T cells is a crucial step in activating antigen-specific CD4+ T cells. The activated CD4+ T cells differentiate into CD4+ T helper cells that can trigger activation and differentiation of CD8+ T cells into cytotoxic T cells. It is known that tumor antigen can be presented by tumor cells in the form of HLA/peptide complexes. However, T-cell-mediated tumor rejection does not occur when tumor cells express HLA-G. Studies with tumor tissues and cell lines have demonstrated that HLA-G-positive or HLA-G-transfected tumor cells are less susceptible to recognition by CD4+ T cells and cytolytic killing by CD8+ cells. How this can occur has intrigued investigators for a long time, and it has now become clear that several mechanisms are involved in HLA-G-induced inactivation of T-cell immunity. For example, it has been reported that interaction between HLA-G and CD4+ T cells via LILRB1 and LILRB2 can reduce production of T-helper 1 (Th1) cytokines including IFN-γ, IL-2 and tumor necrosis factor-α but enhance production of Th2 cytokines including IL3, IL-4 and IL-10, which results in inactivation of cytotoxic T cells and reduction of antitumor antibody secretion.

sHLA-G can also bind to CD8 and induce apoptosis through activation of the Fas/FasL pathway. Bahri et al have reported that HLA-G can block cell cycle progression from G1 to G2/M phase in T cells. A recent study has indicated
that HLA-G1-transfected antigen-presenting cells can reduce CD4+ T-cell proliferation by inducing T-cell anergy or long-term unresponsiveness.57 Other studies have demonstrated that immune tolerance mediated by HLA-G molecules can be achieved by induction of immunosuppressive/regulatory T cells.57–60 These cells have been found to reduce T-cell activation and promote T-cell death, thus facilitating immunosuppression and long-term immune evasion or tolerance. Finally, HLA-G expression can also abolish cytotoxic T-cell-mediated cell lysis through interaction of HLA-E and the CD94/NKG complex. These events lead to almost complete abortion of the immune responses, and as a result, tumor cells are protected from T cell attack.

**Interactions with NK cells**

NK cells are responsible for detecting and eliminating cells that are undergoing malignant transformation. Engineered expression of HLA-G protein in MHC-class-I negative cells has been demonstrated to inhibit cytolysis by NK cells.61–63 LIR-2 and KIR2DL4 on the cell surface of NK cells appear as the main inhibitory receptors that interact with HLA-G, and both receptors, upon HLA-G binding, are thought to mediate inhibitory effects on NK cells. Furthermore, HLA-G might indirectly contribute to suppression of NK-cell-mediated cytolsis through upregulation of HLA-E.64 Besides, sHLA-G has been shown to be involved in producing pro-angiogenic cytokines in NK cells through KIR2DL4,65 which could contribute indirectly to tumor development.

**Interactions with DCs**

DCs play a central role in regulating immune responses and maintaining peripheral tolerance.61,66,67 Previous reports have shown that the interaction between HLA-G and its receptors
(LILRB1 and LILRB2) on DCs results in inactivation of the MHC class II presentation pathway and downregulation of co-stimulatory molecules, B7.1 (CD80) and B7.2 (CD86). IL-12 production and chemokine receptor CCR7 expression on these targeted DCs are also significantly inhibited. All the above effects lead to suppression of DC maturation, which can further contribute to failure or unresponsiveness of T-cell activation and clone expansion in response to tumor-antigen stimulation.

Interactions with B cells

The major function of B cells is to generate and secrete antibodies that directly target novel soluble antigens or antigens on abnormal cells, thus playing a central role in adaptive humoral immunity. Until now, there has been a lack of evidence to show the direct interaction between HLA-G and the surface of B cells. However, the possibility of such interaction exists because of the presence of LILRB1 and LILRB2 on B cells. The biological effects of HLA-G in modulating B-cell functions in human cancer remain to be determined.

New Treatments and Therapeutics Against HLA-G Expression

Since HLA-G expression in tumor tissues is a unique feature that negatively correlates with clinical outcomes, HLA-G accordingly becomes an attractive target for developing new interventions against more invasive and metastatic cancer cells. Given the fact that the main inhibitory effects of HLA-G are mediated by its interaction with lymphocyte immunoglobulin-like receptors, blocking the reaction by antagonistic recombinant proteins or neutralizing antibodies should be beneficial for treating HLA-G-expressing cancer cells. By in vitro allostimulation assays, antibodies against LIR-1 and LIR-2 have successfully restored T-cell proliferation, which indicates the effectiveness of such a therapeutic approach. Similar results can also be found when treating cancer cells with anti-FasL antibodies that block the Fas/FasL pathway through binding of HLA-G to LIR-1 or LIR-2. These data support a potential application of using HLA-G blockers, such as HLA-G neutralizing antibodies or soluble recombinant LILRB1, LIR-2 or Fas, as therapeutic agents to minimize the inhibitory effects of HLA-G molecules.

Conversely, sHLA-G serves as an ideal biomarker for cancer detection in body fluids. The value of HLA-G expression in predicting clinical outcome in certain cancers and the HLA-G tests (e.g. immunohistochemistry or quantitative real-time polymerase chain reaction) are expected to provide oncologists with a new molecular approach to manage their cancer patients better. Anti-HLA-G antibodies could also be utilized for developing a cancer imaging system to monitor the activity and location of tumor cells that have been tolerated by the immune defense system. Target-based chemotherapy can be achieved by using antibody delivery methods to bring the cytotoxic drugs to the more aggressive tumor tissues. Small interfering RNA/small hairpin RNA therapies could be also applied to HLA-G-expressing cells by this antibody-oriented route. Furthermore, enhancing the Th1 cytokine profile in the host by boosting more potent immunomodulators or by immunization with cancer vaccine might also be feasible for changing the microenvironment to reactivate host immune surveillance. More studies need to be conducted to evaluate the efficacy of each method in clinical applications.

Several anticancer drugs have been found to be inducers for cancer cells to express higher levels of HLA-G proteins, which results in tumor evasion of the host immune system. For example, 5-aza-2’-deoxycytidine, a demethylating agent for treating cancer patients during epigenetic therapy, could reactivate expression of HLA-G protein in all the cell lines tested. Similarly, IFN immunotherapy of malignant tumors can cause side effects of immune evasion by upregulating the expression of HLA-G at the tumor sites. Screening of tumor lesions for HLA-G expression might represent a useful strategy to identify the patients who
are likely to benefit from epigenetic and IFN therapy.

Acknowledgments

This study was supported by a research grant from the National Institute of Health (RO1CA103937), Bethesda, MD, USA and a research grant from China Medical University (CMU97-CMC-006), Taichung, Taiwan.

References


