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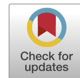
REVIEW ARTICLE

HLA-G and single nucleotide polymorphism (SNP) associations with cancer in African populations: Implications in personal medicine



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Abstract The immune system plays an important role in protecting the body against malignancy. During cancer immunoediting, the immune system can recognize and keep checking the tumor cells by down-expression of some self-molecules or by increasing expression of some novel molecules. However, the microenvironment created in the course of cancer development hampers the immune ability to recognize and destroy the transforming cells. Human Leukocyte Antigen G (HLA-G) is emerging as immune checkpoint molecule produced more by cancer cells to weaken the immune response against them. HLA-G is a non-classical HLA class I molecule which is normally expressed in immune privileged tissues as a soluble or membrane-bound protein. *HLA-G* locus is highly polymorphic in the non-coding 3′ untranslated region (UTR) and in the 5′ upstream regulatory region (5′ URR). HLA-G expression is controlled by polymorphisms located in these regions, and several association studies between these

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polymorphic sites and disease predisposition, response to therapy, and/or HLA-G protein expression have been reported. Various polymorphisms are demonstrated to modulate its expression and this is increasingly finding more significance in cancer biology. This review focuses on the relevance of the *HLA-G* gene and its polymorphisms in cancer development. We highlight population genetics of *HLA-G* as evidence to espouse the need and importance of exploring potential utility of HLA-G in cancer diagnosis, prognosis and immunotherapy in the currently understudied African population.
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Introduction

Cancer remains to be a global problem, affecting both developed and less developed countries. While the overall incidence of cancer is as twice in developed as in developing countries, the mortality rate is higher in developing countries than that in developed countries. This difference has been attributed, in developing countries, to late cancer diagnosis, poor available treatment and bearing a burden of highly fatal cancers.[1]

Cancer pathogenesis involves the expression of various tumor-associated and tumor-specific molecules, which can trigger the immune response.[2,3] Despite the impressive function of immune cells to recognize and destroy cancerous cells, tumors develop some escape mechanisms from immune attack. Both the microenvironments created in the course of tumorigenesis and some molecules expressed by tumor cells tend to suppress immune cells' response to such transformed cells.[4–6] Human Leukocyte Antigen G (HLA-G) has recently gained popularity as among the immunosuppressive molecules expressed by various cancer cells contributing to their immune escape.[7–11]

HLA-G is a non-classical HLA class I molecule, which is restrictively expressed by cells in immune privileged tissues under normal physiological condition.[12] However, its expression is upregulated in other cells under some special physiological or pathological conditions. In pregnant women, the trophoblast cells existing at maternal–fetal interface express a high level of HLA-G, which physiologically protect the semi-allogeneic fetus against potential maternal immune attack.[13] Some pathological conditions triggering high-level expression of HLA-G include tumorigenesis, inflammatory diseases and some viral infections,[14] (Fig. 1).

The HLA-G molecule has seven isoforms; four isoforms are membrane-bound while the other three are secretory ones.[15] HLA-G interacts with inhibitory receptors on immune cells such as immunoglobulin-like transcript (ILT) and killer cell immunoglobulin-like receptors (KIR) exerting its immunosuppression effects, such as inhibiting cytotoxic ability and induction of apoptosis.[16–18]

Because HLA-G expression is induced in the course of tumorigenesis, it is considered to serve as a biomarker for cancer and a potential target for cancer immunotherapy. Soluble HLA-G (sHLA-G) has been extensively studied in the context of the clinicopathological status of different carcinomas,[19–22] and recently its power to discriminate cancer patients from healthy individuals has been reported.[19,20,23–26] The sHLA-G can be derived from some subsets of immune cells,[27] tumor cells and tumor infiltrating cells.[7] Also, some studies have demonstrated the promising potential of targeting HLA-G for the therapeutic purpose.[28–30]

It is becoming evident that, genetic variations undergird the development of most complex diseases such as cancer. The most type of genetic variation among people are the Single Nucleotide Polymorphisms (SNPs) characterized by difference in one nucleotide at a specific point in the genome. The occurrence of SNPs in the coding region of the gene may affect the conformational and functional nature of the corresponding protein. Also, the existence of SNPs in the regulatory region may have an effect on the expression level of the respective genes and subsequently affecting the amount of produced proteins. Consequently, such effect may lead to impairments of both cellular and physiological roles of the affected proteins and hence becoming pathologically significant. SNPs are gaining significance in understanding individual susceptibility to wide range of diseases particularly cancer and response to drugs.[31]

The *HLA-G* gene bears some genetic variations that influence its expression. Of particular significance are the polymorphisms found in the non-coding regions of *HLA-G* gene which modulate the expression of HLA-G through affecting the affinity of regulatory sequence to transcription factors and stability of mRNA.[32–36] Because HLA-G expression tends to suppress the immune response to tumor cells, the aberrant expression of HLA-G polymorphic variants are plausibly associated with cancer susceptibility and progression.

Albeit impressive findings reported by various population-based studies on the relationship between HLA-G biology and cancer, some controversies still exist over how the molecule is implicated in cancer development. And so far, the African population characterized by high genetic diversity is much understudied in the context of the relationship between HLA-G and cancer. Extending more research on oncological implication of HLA-G to ethnically diverse populations is of paramount importance in understanding the potential clinical utility of the molecule.

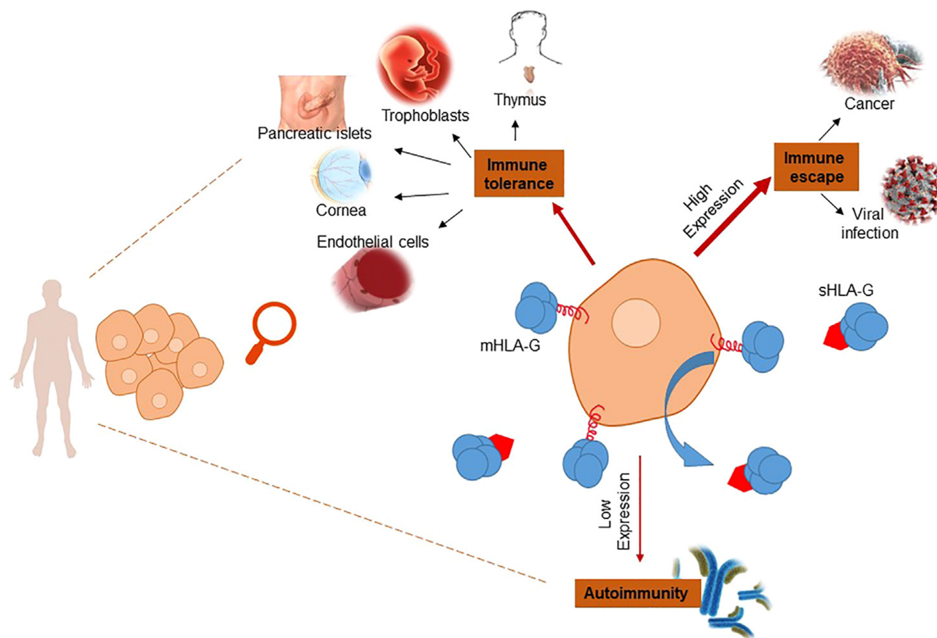

Figure 1 HLA-G and immunotolerance. Under normal physiological conditions, HLA-G is more expressed in immune privileged sites such as cornea, pancreatic islets, endothelial cells, thymus, and erythroblasts and in hematopoietic cell lineage. Its expression is upregulated in pathological conditions such as viral infections and cancer. Its downregulated expression is implicated in autoimmune diseases. HLA-G1 to -G4 are membrane-bound because they possess transmembrane (Tm) region anchoring them to the membrane and cytoplasmic (Cyt) domain. HLA-G5 to -G7 are soluble ones as they lack transmembrane domain. Intron (I) 2 intervening exons 2 and 3, and intron 4 intervening exons 4 and 5 contain stop codons (red dots) and are retained in mRNAs for HLA-G5, -G6 (intron 4) and -G7 (intron 2). Termination of translation at these stop codons makes some parts of introns to be incorporated into HLA-G5, -G6 and -G7 as short tails. α_1, α_2 and α_3 are extracellular domains expressed from exons 2, 3 and 4, respectively. UTR: untranslated region; mHLA-G: membrane-bound HLA-G; sHLA-G: soluble HLA-G.

Basic biology of human leukocyte antigen-G (HLA-G)

Human leukocyte antigens are cell-surface proteins which were first discovered in the 1930's by Peter Gorer as the antigens which take place in rejection of transplanted tissues.[37] These antigens are encoded by genes clustered within Major Histocompatibility Complex (*MHC*) at Chromosome 6 in humans.[38] MHC complex is divided into three regions, *MHC-I*, *MHC-II* and *MHC-III*. *MHC-I* and *-II* encode protein responsible for the presentation of antigenic peptides on the cell surface, which elicit an immune response. MHC-I molecules are expressed by all nucleated cells, and they present antigenic peptides derived from endogenous proteins. In contrast, MHC-II molecules are exclusively expressed by antigen-presenting cells (APCs) which present antigenic peptide derived from endocytosed exogenous proteins.[39] *MHC-III* genes encode various secretory molecules essential in immune system function, such as components of the complement system, heat shock proteins and some lymphokines.[38]

The HLA class I molecules can be divided into classical and non-classical HLA. The antigenic presentation function pinpointed above is carried out by classical HLA class I.[40] Unlike classical HLA class I, non-classical class I HLA molecules are restrictively expressed in some specific tissues and display functions differently from that of classical HLA class I molecules. Non-classical HLA class I molecules include HLA-G, HLA-E and HLA-F. The immunosuppressive function of HLA-G is important in protecting a developing semi-allogeneic fetus against maternal immune attack[13] and in mediating the development of pathological conditions associated with impaired or reduced immune efficiency such as cancer, viral infections and autoimmunity.

According to IMGT/HLA notation, *HLA-G* gene consists of seven introns and eight exons, though its primary transcript contains seven exons. Exons 2, 3 and 4 correspond to extracellular domains α_1, α_2 and α_3 in the expressed protein, respectively. While exon 5 encodes peptides that make up a transmembrane region of HLA-G protein, exon 6 contains information for a short cytoplasmic tail composed of six amino acids. Exon 1 encodes peptides that act as signals for directing the movement of HLA-G to the right site. Exon 7 is not transcribed into a primary transcript, and exon 8 makes the 3′-untranslated region of HLA-G mRNA (Fig. 2).[15] *HLA-G* gene is less polymorphic compared to classical HLA class I genes, only 82 *HLA-G* alleles encoding 22 full-lengths (IMGT/HLA-G Release 3.44.0, April, 2021) are currently known.

The post-transcriptional processing of HLA-G primary transcript can, by alternative splicing, produce seven isoforms of HLA-G protein, HLA-G1, HLA-G2, HLA-G3, HLA-G4, HLA-G5, HLA-G6 and HLA-G7. Isoforms HLA-G1 to -G4 are membrane-bound due to the containment of transmembrane and cytoplasmic domains, but HLA-G5 to -G7 lack transmembrane domains and hence exist in soluble

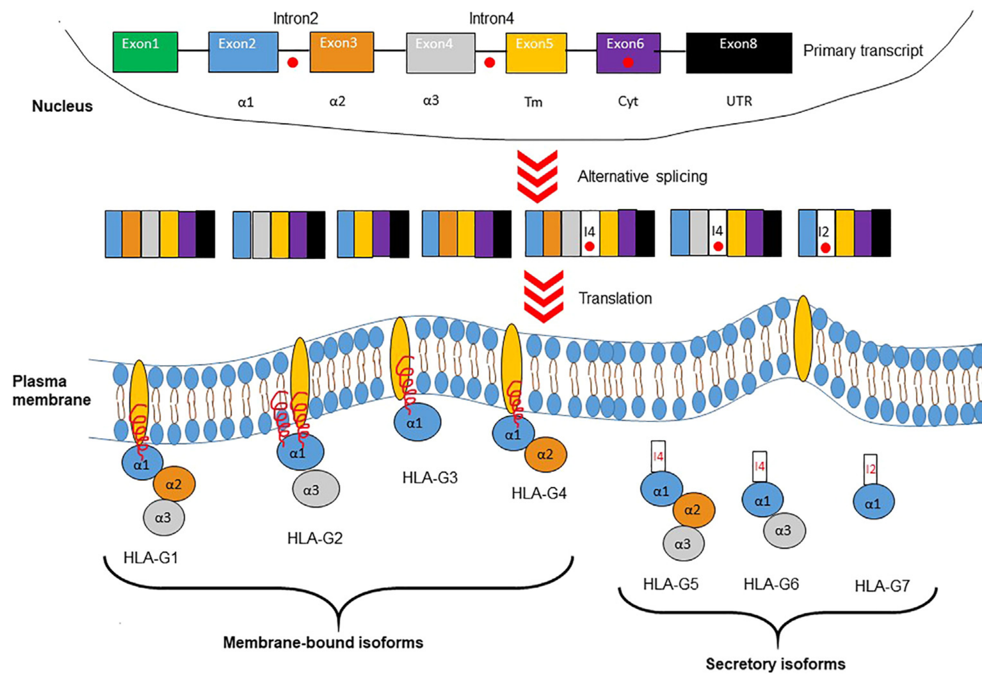

Figure 2 HLA-G isoforms translated from alternatively spliced exons in HLA-G primary transcript. HLA-G1 to -G4 are membrane-bound because they possess transmembrane (Tm) region anchoring them to the membrane and cytoplasmic (Cyt) domain. HLA-G5 to -G7 are soluble ones as they lack transmembrane domain. Intron (I) 2 intervening exons (E) 2 and 3, and intron 4 intervening exons 4 and 5 contain stop codons (red dots) and are retained in mRNAs for HLA-G5, -G6 (intron 4) and -G7 (intron 2). Termination of translation at these stop codons makes some parts of introns to be incorporated into HLA-G5, -G6 and -G7 as short tails. α_1, α_2 and α_3 are extracellular domains expressed from exons 2, 3 and 4, respectively.

forms.[41] HLA-G1 has all three (α_1, α_2 and α_3) extracellular domains, HLA-G2 lacks α_2 and hence has two extracellular domains, HLA-G3 lacks domains α_2 and α_3 and hence has one extracellular domain, and HLA-G4 lacks domain α_3 only, and so it has two domains. HLA-G5, -G6 and -G7 are soluble forms of membrane-bound HLA-G1, -G2 and -G2 respectively as they possess respective extracellular domain (s), but lack transmembrane and cytoplasmic domains counterparts. Additionally, HLA-G5, -G6, and -G7 contains small translated parts of introns 4, 4 and 2, respectively.[15]

Soluble forms of HLA-G can also be derived from membrane-bound HLA-G shed from their cell membranes.[41] The shedding process is catalyzed by proteolytic cleavage of HLA-G from the cell membrane by metalloproteases.[42,43] Some other soluble forms of HLA-G exist as secretory isoforms enclosed into vesicles.[44] The vesicular form of soluble HLA-G can shuttle HLA-G between cells as it is capable of fusing with the plasma membrane of cells. A cell not expressing membrane-bound HLA-G can take up HLA-G expressed on the other cell through a process called trogocytosis.[45]

To be able to present antigenic peptides, HLA class I molecules associate with β_2-microglobulin (β_2M) which act as a stabilizer. Only HLA-G1 and HLA-G5 possessing all three extracellular domains can bind β_2M protein, and some researchers suggest that HLA-G1 is potentially capable of presenting antigenic peptides.[46] Apart from those forms of HLA-G, the molecules can exist as dimers or even trimer. Dimerization is made possible due to the existence of some cysteine residues in α_1 and α_2 domains which can form disulfide bond between two or more HLA-G molecules.[47]

Le Roux et al reported the possibility of the existence of some new HLA-G isoforms other than the currently known seven isoforms.[48] By working with clear cell renal cell carcinoma, immunoassay and transcriptome analysis of HLA-G revealed the existence of HLA-G isoforms lacking α_1 domain, $\alpha_1\alpha_2$ domains, transmembrane and cytoplasmic domains and having extended 5’ part. It is important to explore more about this possibility, as some novel HLA-G isoforms might find essential utility in diagnosis or treatment of cancer or other pathological conditions.

Immunomodulatory functions of HLA-G molecule

Under both physiological and pathological conditions, it has been demonstrated that HLA-G functions by inhibiting normal cellular functions of immune cells. This function is responsible for normal physiological immune tolerance and viral/altered cells immune escape in pathological conditions. The immunosuppressive function of HLA-G was firstly studied in search of its role in pregnancy maintenance as its expression was observed to be upregulated in trophoblasts. Rouas-Freiss et al utilized monoclonal antibody specific to HLA-G on trophoblast cells co-cultured with semi-allogeneic natural killer (NK) cells.[13] NK cells in anti-HLA-G treated cell culture showed higher cytotoxic activity compared to those in a culture not treated with the anti-

HLA-G antibody. Anti-HLA-G neutralized the activity of HLA-G, allowing NK cell cytotoxicity to take place.

As the fetus carries some paternally derived molecules which can be recognized by maternal immune system as antigens, HLA-G induces the maternal immunotolerance to this semi-allogeneic creature, sparing it from immune cells mediated destruction.[15] Some studies have demonstrated this fetus protective role of HLA-G, as downregulation of HLA-G in trophoblasts during pregnancy is associated with miscarriage. The recurrence of miscarriages in some women is associated with the genetic variants of *HLA-G* gene expressing low amount or no HLA-G molecules.[49,50]

Other immunosuppressive role of HLA-G has been extended to autoimmune diseases and viral infections pathogenesis. In autoimmune diseases, the immunotolerance to the self-molecules is disrupted and the self-molecules are recognized and destructed by immune cells. For instance, downregulation of HLA-G expression in rheumatoid arthritis patients has been observed by Verbruggen et al[51] and Gautam et al.[52] Virally infected cells normally present the viral derived antigens on their surfaces, eliciting the immune response, which subsequently kills virus bearing cells. However, many viruses exploit different mechanisms to escape from immune recognition, and upregulation of HLA-G has been observed in patients infected with viruses such as HIV,[53] and human cytomegalovirus.[54]

The immunosuppressive role of HLA-G has been extensively studied in cancer development due to its increased presence in tumor tissues and patients' circulatory system. Both *in vitro* and *in vivo* studies have demonstrated that HLA-G in tumor cells inhibits the immune response. Paul et al[55] demonstrated the protective role of HLA-G on IGR melanoma cell lines against NK cell cytotoxicity. NK cells co-cultured with melanoma cell lines expressing an upregulated level of HLA-G exhibited poor cytotoxicity against these allogeneic cells. Also, Kim et al[52] showed that activation of a subset of $CD8^+$ T cells expressing Leukocyte Ig-like receptor B1 (LILRB1), a receptor for HLA-G and responsive to Bispecific T cell engager (BiTE) molecule is inhibited when the receptor is engaged with HLA-G ligand on tumor cell. Blockade of this receptor along with programmed cell death protein 1 (PD1) resulted into pronounced activation of the $CD8^+$ T cells.

The similar effect has been demonstrated by Loumagne et al.[56] In this *in vivo* study, the immunosuppressive role of soluble HLA-G was investigated by transfecting some tumor cell lines with immunogenic $h\beta_2M$ molecule only and others with $h\beta_2M$ and HLA-G5. After injecting the transfected tumor cells in the immunocompetent mouse, tumor cells transfected with $h\beta_2M$ only were rejected by the mouse immune system. However, the tumor cells transfected with both $h\beta_2M$ and HLA-G5 could not be rejected by the mouse, indicating the protective role of HLA-G5 to tumor cells against host immune attack. HLA-G mRNA interference in tumor cells by short hairpin RNA (shRNA) also improved their susceptibility to NK cells cytotoxicity.[57]

HLA-G can exert its immunosuppressive effect directly or indirectly. Direct exertion is achieved by interaction of HLA-G molecule with specific receptors on immune cells which transmits an inhibitory signal.[58] The notable inhibitory receptors for HLA-G are ILT4, ILT2 and KIR2DL4 which are widely expressed by different immune cells. Cells expressing ILT2 include B cells, some T and NK cells, monocytes/macrophages and dendritic cells. Dendritic cells, monocytes/macrophages and neutrophils express ILT4 while KIR2DL4 is expressed by some T cells and NK cells.[15] The receptors transmit the inhibitory signal through Immuno-receptor tyrosine based inhibitory motif (ITIM). HLA-G and ILT4 have been reported to be co-expressed in colorectal cancer (CRC) tissues and their interaction contributes to the progression of CRC.[59] Some evidence shows that downregulation or blocking of ILT2 on NK and $CD8^+$ T cells rescues their cytotoxic effect against tumor cells.[6,60]

The binding of HLA-G to most receptors is through α_3 domain which is present in most membrane-bound and soluble forms of HLA-G.[61] This implies that soluble HLA-G may have a systemic effect to immune system impairment. Interaction of HLA-G with ILT2 on B cells inhibits both naïve and memory B cells' proliferation, differentiation and secretion of immunoglobulin by arresting the cell at G_0/G_1 of the cell cycle.[62] Some soluble forms of HLA class I molecules, including HLA-G induce apoptosis of $CD8^+$ T and NK cells by interacting with CD8 molecule and upregulating the expression of surface and soluble first apoptosis signal ligand (FasL).[63] Recently, Schwich et al have demonstrated that activation of $CD8^+$ T cells previously primed with sHLA-G results into immunosuppressive phenotype of the T cells characterized by increased expression of ILT2 and immune checkpoint molecules including Cytotoxic T Lymphocyte Antigen 4 (CTLA-4), PD1, T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) and CD95.[64]

The indirect way by which HLA-G suppresses immune cells function is through activation of immune cells differentiation to regulatory immune cells such as regulatory T cells, regulatory B cells, myeloid-derived suppressor cells (MDSCs) and Dendritic cells-10 (DC-10).[65,66] All these regulatory cells tend to suppress the normal cellular functioning of immune cells. Furthermore, HLA-G indirectly impairs the immune cells function through trogocytosis.[45,67] Subsequently, trogocytosis induces cellular anergy and affects the cytokine production profile and expression of receptors. However, Ostapchuk et al[68] demonstrated that NK cells expressing HLA-G modulate negatively the cytotoxicity of other NK cells not expressing HLA-G through direct cell—cell contact, and not through altered cytokines produced by HLA-G bearing cells.

Diagnostic, prognostic and therapeutic implications of HLA-G expression in malignancies

The increased expression of HLA-G in both solid and hematological tumors have been confirmed in various studies. Elevated expression of HLA-G has been observed in tumor lesions as membrane-bound HLA-G. For example, membrane-bound HLA-G has been found in the lesions of breast,[26] cervical,[69] endometrial,[11,70] vulva squamous cell[71] and lip squamous cell[72] cancers. The secretory form of HLA-G has also been found in body fluids like blood, ascites and saliva as sHLA-G.[19,24,73] Higher expression of HLA-G in cancerous cells than in pre-cancerous counterparts reflects an influence of HLA-G on cancer progression.[19,69]

In various cancers, the sHLA-G found in the circulatory system has shown to have diagnostic and prognostic significances. In most studies, plasma sHLA-G levels are reported to be significantly higher in the malignant tumor patients than in controls.[24,25,74,75] This difference in sHLA-G levels has been investigated whether it can truly be useful to discriminate tumor patients from normal people, or benign tumors from malignant tumors. Selected findings on such discriminatory utility of sHLA-G are summarized in Table 1.

As presented in Table 1, most studies investigating the diagnostic potential of sHLA-G in different types of cancer report its good diagnostic performance (Area Under Receiver Operating Characteristic Curve (AU-ROC) > 0.7). Some other studies even compared the diagnostic capability of sHLA-G with other common used tumor markers. The sHLA-G was found to significantly outperform Cancer antigen (CA) and Carcinoembryonic (CEA) as tumor markers for breast,[25] gastric,[24] colorectal, esophageal and lung cancer.[23] Heidari et al[76] compared the diagnostic significance of sHLA-G against conventional PSA marker for prostate cancer diagnosis. The sHLA-G was shown to have a better performance than PSA, and the authors recommended sHLA-G to be used as a supplementary biomarker to PSA in prostate cancer diagnosis.

The potential of sHLA-G as a biomarker for cancer progression has also been investigated[19] and was reported to have a good discriminatory utility between benign and malignant tumors among primary liver, lung, colon, ampullary, renal, cervical, pancreatic, ovarian cancer and non-Hodgkin lymphoma as malignant tumors, and liver cirrhosis and tuberculosis peritonitis as benign tumors. Also, Jeong et al[25] showed the diagnostic performance of sHLA-G to differentiate metastasized cancer from other stages. However, in most studies, the sHLA-G levels are not consistently associated with specific tumor stage/grade.

Some studies have associated increased sHLA-G expression with poor prognosis of cancer.[77] However, contrary results have been reported from Rutten et al[78] study where increased sHLA-G levels were associated with prolonged survival among high grade ovarian carcinoma patients. Moreover, in B cell neoplasms, HLA-G expression is normally associated with better prognosis and this has been attributed to the inhibitory effect of HLA-G exerted to the malignant B cells upon interacting with ILT2 receptor which leads to the reduced proliferative ability of B cells, and hence improving the clinical condition.[79] Yet, the findings from the study by Yong et al[80] in non-Hodgkin lymphoma patients showed that sHLA-G is a poor prognostic marker in clinical settings.

Some studies have investigated the usefulness of HLA-G in monitoring the patient's response to cancer treatment. The study conducted by Sayed et al[81] reported a significant decrease in sHLA-G levels among mastectomized breast

Table 1 Diagnostic performance of sHLA-G in some selected studies on cancer.

Condition	Discriminatory Utility	Sample	AU-ROC	COV	S_N (%)	S_P (%)	Population	Ref
Breast cancer	Patients *vs.* Control	plasma	0.89	19.4 U/ml	92.5	70	Korea	25
NSCLC	Patients *vs.* Control	plasma	0.82	24.9 U/ml	52.8	100	Caucasian	75
Gastric cancer	Patients *vs.* Control	plasma	0.730	84 U/ml	27.2	95.4	China	24
Gastric cancer	Patients *vs.* Control	plasma	0.70	—	—	—	China	117
Carcinomas	Malignant *vs.* Benign	ascites	0.957	19.6 μg/L	87.5	100	China	19
Breast cancer	Metastasis *vs.* Other stages	plasma	0.79	147.7 U/ml	88.9	69.0	Korea	25
Colorectal cancer	Early stages patients *vs.* Control	plasma	0.97	49 U/ml	94	100	China	23
Gastric cancer	Early stages patients *vs.* Control	plasma	0.91	49 U/ml	85	100	China	23
Esophageal cancer	Early stages patients *vs.* Control	plasma	0.98	49 U/ml	91	100	China	23
NSCLC	Early stage patients *vs.* Control	plasma	0.8	49 U/ml	51	100	China	23
Breast cancer	Patients *vs.* Control	plasma	0.935	68.82 U/ml	65.9	100	China	26
Breast cancer	Patients *vs.* Control	plasma	0.95	0.54 μg/L	88.1	100	China	20
Cervical cancer	Premalignant *vs.* Malignant patients	plasma	0.694	108.2 U/ml	73.3	67.7	China	69
Gastrointestinal cancer	Patients *vs.* Control	plasma	0.752	57.85 U/ml	89	62	Iran	74

Abbreviations: AU-ROC, area under receiver operating characteristic curve; COV, cut-off value; SN, sensitivity; SP, specificity; NSCLS, non-small cell lung cancer; Ref, reference.

cancer patients undertaking adjuvant therapy after 12 months of follow up. Though, the few patients whose sHLA-G levels remained high were found to have had their cancers metastasized. Similarly, in our recent study,[82] we found a significantly lower amount of sHLA-G among breast cancer patients who had undergone mastectomy as compared to those who had not. Rutten et al[78] found a significant decline in sHLA-G levels following chemotherapy treatment of high-grade ovarian cancer, and the sHLA-G increased to almost the initial levels following recurrence. Similarly, a significant decrease in serum sHLA-G levels following therapy among head and neck squamous cell carcinoma (HNSCC) patients has recently been reported by Agnihotri et al.[83] These changes in sHLA-G levels in response to medical interventions carry the potential of being translated in monitoring treatment response among cancer patients.

The abnormal rise of HLA-G expression in tumor tissues makes it a potential target for immunotherapy. In the course of tumorigenesis, tumor cells downregulate the expression of classical HLA class I, making them unable to present tumor antigen peptide on their surfaces to elicit T cells immune response.[84] This downregulated expression of classical HLA class I deprives these cells of some self-molecules, hence becoming susceptible to NK cytolytic attack. The upregulation of immunosuppressive molecule HLA-G by tumor cells circumvents this recognition and lysis by NK cells, making tumor progression possible.[55] Therefore, an approach capable of downregulating expression of HLA-G by tumor cells could improve the efficacy of immunotherapy.[85,86]

The approaches to intervene in the interaction between HLA-G molecule on tumor cells and the respective receptors on immune cells have demonstrated an improved immune response to tumor cells. Blocking tumor HLA-G with monoclonal antibodies increased tumor lysis in mice injected with M8-HLA-G1 cell lines.[87] Also, suppression of formation of regulatory T cells ($CD4^+/CD25^{high}/Foxp3^+$ Tregs) and subsequent proliferation of allogeneic T cells was observed upon neutralizing sHLA-G molecule.[88] Downregulation of HLA-G mediated by siRNA improved cytolytic ability of NK cells against human hepatocellular carcinoma cell line.[30]

Ishibashi et al[89] investigated the possibility of priming HLA class II-restricted immune response to tumor cells by using the peptide derived from HLA-G. Upon stimulating $CD4^+$ Helper T Lymphocytes (HTLs) with HLA-G derived peptide, they were able to recognize tumor cells bearing HLA-G and mediate immune response to tumor cells. The response is even elevated when HLA-G expression by tumor cells is further upregulated by treating tumor cells with DNA methyl transferase (DNMT) inhibitor (5-aza-2′-deoxycytidine). Strangely, this study shows that $CD4^+$ HTLs possess the cytolytic ability as they seemed to lyse tumor cells bearing the HLA-G molecule.

Based on the studies conducted so far, HLA-G seems to be a promising biomarker for diagnosis and prognosis in different types of cancer. The supplementary usage of this molecule to other conventional biomarkers for respective types of cancer could potentially improve the diagnostic and prognostic outcome. However, the body of knowledge has limited findings of that kind from many African ethnic groups. More approaches in ethnically diverse populations are needed to investigate the possibility of harnessing the HLA-G biology to improve cancer diagnosis, prognosis and treatment.

HLA-G polymorphisms, expression and population genetics: rationale for more cancer-related HLA-G studies in African population

HLA-G locus, different than classical *HLA* class I gene, is highly polymorphic in the non-coding 3′ untranslated region (UTR) and in the 5′ upstream regulatory region (5′ URR). Variations in 3′UTR region influence HLA-G expression by modifying mRNA stability, and the variations in 5′URR affect the binding of transcription factors to their cognate regulatory sequences.[90] Thereby, the genetic variations in the *HLA-G* gene affect its expression at both transcriptional and post-transcriptional levels. Owing to the significance of elevated expression of HLA-G in cancer, it is important to explore the high-producers genetic variants of HLA-G which can serve as genetic determinants of cancer susceptibility and progression.

The 5′URR of *HLA-G* gene contains at least 35 Single Nucleotide Polymorphisms (SNPs)[91] which may influence its transcriptional level by affecting the binding of transcriptional factors to regulatory elements. Examples of such SNPs include −666G/T, −689A/G, −716T/G, −725C/G/T, −762C/T, −810C/T, −964G/A, −990G/A, −1140A/T, −1179A/G and −1306G/A.[92] The −725C/G/T SNP is among the most commonly studied SNPs, and the *in vitro* study by Ober et al[49] demonstrated higher promoter activity of −725G allele variant than the −725C and −725T alleles. These findings brings some controversy over the role of high expression of HLA-G in pregnancy maintenance, as some studies have associated −725G allele with miscarriage.[93] However, SNPs −725C/G/T and −716T/C have been studied in relation to non-small cell lung cancer and no association was determined.[94]

Some notable polymorphisms in 3′UTR include 14-bp insertion/deletion (14-Indel) at position +2961, +3001C/T, +3003T/C, +3010C/G, +3027A/C, +3032G/C, +3035C/T, +3052C/T, +3092G/T, +3107C/G, +3111G/A, +3142G/C, +3187A/G, +3196C/G and +3227G/A. These polymorphisms exist in seven most frequent haplotypes (Fig. 3) and their worldwide frequencies distribution have been reported by Sabbagh et al (Fig. 4).[95] Haplotypes UTR-1 and UTR-2 are the most prevalent haplotypes conserved in all worldwide populations differing at polymorphic site +2961, +3010, +3142, +3187 and +3196.[95]

UTR-1 is theoretically considered to be a higher producer of HLA-G than UTR-2. The results congruent to the theory have been reported in the two independent population studies where UTR-2 haplotype was found to be associated with lower expression of sHLA-G in Malian population,[96] and UTR-1 being associated with higher expression of sHLA-G in Brazilian and French population.[36] The association of UTR-1 with high HLA-G expression is further supported by the *in vitro* study by Poras et al, which utilized luciferase activity assay to assess the impact of UTR haplotypes on HLA-G expression. This study revealed

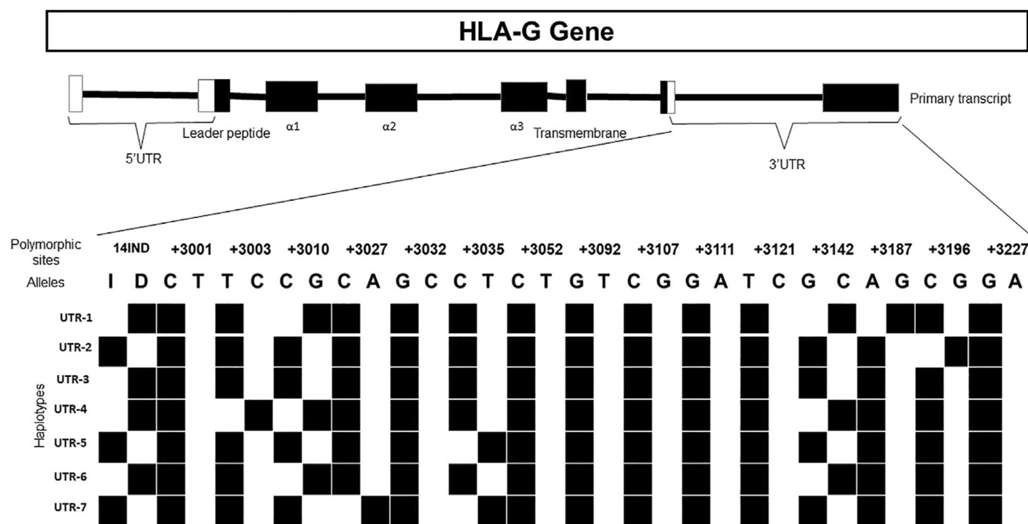

Figure 3 Common variation sites and haplotypes in 3′UTR of HLA-G gene based on 21 global populations. The shaded squares indicate the presence of allelic form in the respective haplotypes. The allelic variants in the haplotypes affect the expression of HLA-G. UTR-1 and UTR-2 are the most prevalent haplotypes, differing at five polymorphic positions. "14IND" stands for 14-bp Insertion/deletion; "I" stands for 14-bp **I**nsertion; "D" stands for 14-bp **D**eletion. The gene structure is according to NCBI notation.

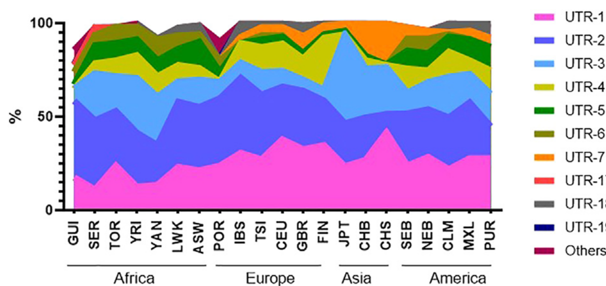

Figure 4 Global distribution of frequencies of HLA-G 3′UTR haplotypes (adapted from Sabbagh et al[95]). There are variations in distribution of some haplotypes across four continents. UTR-7 is almost non-existent in African population while UTR-19 is absent in almost other continents but Africa. Abbreviations: ASW, People from southwestern United States with African ancestry; CEU, Utah residents with Northern and Western European ancestry; CHB, Han Chinese from Beijing; CHS, Han Chinese from South China; CLM, Colombians from Medellı'n, Colombia; FIN, Finnish from Finland; GBR, British from England and Scotland; GUI, Natives of Guinea-Bissau; IBS, Iberian population from Spain; JPT, Japanese from Tokyo, Japan; LWK, Luhya from Webuye, Kenya; MXL, People of Mexican ancestry from Los Angeles; NEB, North eastern Brazilians from Recife, Pernambuco, Brazil; POR, Portuguese; PUR, Puerto Ricans from Puerto Rico; SEB, Southeastern Brazilians from RibeiraoPreto, Sao Paulo, Brazil; SER, Serer from Niakhar, Senegal; TOR, Tori from Tori-Bossito, Benin; TSI, Toscani from Italy; YAN, Yansi from Bandundu, Democratic Republic of Congo; YRI, Yoruba from Ibadan, Nigeria.

further that UTR-5 and UTR-7 are associated with low expression of HLA-G as they impacted luciferase activity the most.[35]

The differential mRNA repertoire present in different cells has been suggested to be the reason behind the conflicting results. Not only is the stability of HLA-G mRNA affected by the polymorphism in the 3′UTR, but also the nature of the available micro-RNAs (miRNAs) is. While most miRNAs, which are well known to mediate the degradation of HLA-G mRNA, bind with high affinity to 3′UTR region in a polymorphic variant-specific manner, some others such as miR-139-3p bind to non-specific regions of HLA-G mRNAs regardless of their polymorphic nature.[97] It is therefore recommended for the studies exploring associations of *HLA-G* polymorphism with both HLA-G expression and pathological conditions to take into account of cellular miRNA expressivity.

Most of studies have focused on the relevance of polymorphisms in the regulatory regions to cancer susceptibility, paying little attention to the relative less polymorphic coding region of *HLA-G* gene. Furthermore, more efforts have been devoted to understanding the role of polymorphisms in the 3′UTR region in cancer and other disease conditions as reflected in Table 2. It is important to consider the polymorphisms in both 5′UUR, coding and 3′UTR regions while investigating the relationship between *HLA-G* polymorphism and disease susceptibility, as some haplotypes in both regions have been found to be in linkage disequilibrium.[98,99]

Since HLA-G is a molecule protecting the cancer cells against the anti-tumor immune system, it is expected that *HLA-G* polymorphic variants with high expression of HLA-G to be associated with cancer susceptibility and progression. Figueiredo-Feitosa et al[100] confirmed this among thyroid cancer patients where 3′UTR genetic variants associated with high expression of HLA-G were more prevalent in patients and were associated with poor prognosis. The study by Schwich et al[101] reported prognostic utility of *HLA-G* 3′UTR haplotypes and individual SNPs among epithelial-ovarian cancer patients. In this study, haplotypes UTR-1 and UTR-2, and SNP genotypes +3187G/G and +3196G/G were associated with metastasis and circulating

Table 2 Prognostic and predictive utility of selected HLA-G polymorphisms for cancers.

Polymorphic sites	Cancer	Genotyping method	Population	Year	Potential utility	Ref
Nucleotides encompassing exons 2 and 4 in coding region	Non-small-cell lung cancer (NSCLC)	Sequencing	Tunisia	2016	Allelic variants G*010101, G*010401, G*0105N and G *0106 could serve as independent risk factors for NSCLC	75
SNPs in 3′UTR	Colorectal cancer (CRC)	Sequencing	Italy	2017	SNPs +3196 G/C, 14-Indel and UTR-2 haplotype seem to be predictive markers for FOLFOX4 toxicity in grade 3 and 4 CRC patients	118
SNPs in exons 2 and 3 of HLA-G coding region	Gastric adenocarcinoma (GAC)	PCR-RFLP	Iran	2016	SNPs in HLA-G gene could determine the onset and outcome of GAC	119
+3142G/C	Breast cancer (BC)	PCR-RFLP	Tunisia	2016	SNP +3142G/C could serve as a genetic marker for BC susceptibility	120
SNPs in 3′UTR	Thyroid cancer	Sequencing	Brazil	2017	Some SNPs in 3′UTR associated with high expression of HLA-G are potential markers for differentiated thyroid tumors	100
SNPs in 3′UTR	Colorectal cancer	Sequencing	Italy	2016	SNPs +3196G/C, 14-Indel, and haplotype UTR-2 are potential genetic markers for increased CRC susceptibility	109
SNPs in 3′UTR	Prostate cancer	Sequencing	Brazil	2016	SNP +3003T/C could be used as a tag for prostate cancer risk	108
14-Indel	Breast cancer	PCR + Electrophoresis	Iran	2015	14-Indel is a potential risk factor for BC progression	105
14-Indel	Breast cancer	PCR + Electrophoresis	Korea	2014	14-Indel could serve as a genetic risk factor for BC susceptibility	25
SNPs in 3′UTR	Breast cancer	Taq Man assay	Tunisia	2019	SNP +3142G/C is a potential genetic marker for increased BC susceptibility	103
SNPs in promoter region	NSCLC	Temperature-gradient gel electrophoresis	Poland	2014	SNP -725C > G > T could be used as a marker for lymph node metastasis in NSCLC	121
+3142G/C and 14-Indel	Cervico-vaginal cancer	PCR + Gel electrophoresis	Brazil	2013	+3142 G/C and 14-Indel are potential markers for increased risk of developing cervical cancer	122
−725C > G > T and 14-Indel	Diffuse Large B Cell Lymphoma (DLBCL)	PCR Sequencing	Poland	2015	The SNPs are potentially capable of predicting overall survival of DLBCL patients	123
14-Indel	Post-heart transplant cancers	MassARRAY	Canada	2020	14-bp insertion is a potential predictor of cancer after heart transplantation	124

cancer cells while good clinical outcomes were found to be conferred by haplotypes UTR-5 and UTR-7 and individual SNP variant +3035T. This finding supports the idea that UTR-5 and UTR-7 are related with low expression of HLA-G.[35] The potential clinical utility of some polymorphic variants for cancer is presented in Table 2.

As it is the case in the studies looking for the relationship between *HLA-G* polymorphisms and HLA-G expression, the conflicting results also exist in the studies exploring the relationship between *HLA-G* polymorphism and cancer susceptibility and progression. 14-Indel polymorphism has been demonstrated to affect the stability of HLA-G mRNA.[102] The impact of allelic forms and genotypes of 14-Indel polymorphism on pathological conditions thought to be mediated by elevation of HLA-G expression is controversial, with some researchers reporting associations[25,103] while others

reporting lack of associations.[104,105] The recent meta-analysis by de Almeida et al showed the association of 14-bp deletion allele with increased susceptibility to breast cancer.[106]

Moreover, the *HLA-G* 3′UTR +3142G > C polymorphism is reported to play a pivotal role in the regulation of HLA-G expression by influencing the binding of specific microRNAs (miR-148a, miR-148b and miR-152), and by affecting the stability of the HLA-G mRNA.[107] In agreement, +3142C allele and +3142C/C genotype have been shown to associate with more risk to develop breast cancer in Tunisian population.[103] Besides, the +3142G allele was reported to be associated with prostate cancer.[108] Lack of association between +3142G/C polymorphism and colorectal cancer was also described.[109] Furthermore, the *in vitro* study by Manaster et al[110] demonstrated that +3142G/C polymorphism does not affect HLA-G mRNA targeting by miRNAs.

Amid these conflicting results, it is important to extend cancer-related HLA-G studies to the currently understudied populations, particularly African population rich in genetic diversity. Few African based studies have been conducted on the implication of HLA-G in infectious diseases such as Malaria,[111] Trypanosomiasis,[112] HIV,[113] and hookworm infection[114] while neglecting cancer. The African studies investigating the relevance of HLA-G in cancer have been performed mostly in Tunisian and Egyptian populations which are ethnically different from the larger black population characterizing Africa continent.

It is particularly compelling to study the oncological implication of HLA-G in African population due to the existence of different genetic structures of *HLA-G* between African population and others worldwide. In the comprehensive study on global variations pattern of *HLA-G* gene by Sabbagh et al,[95] the African population was reported to have the highest number of distinct *HLA-G* 3′UTR haplotypes (as many as 35 haplotypes) as compared to other populations worldwide. Some haplotypes such as UTR-7 were almost non-existent in the African population as compared to populations in other continents.

The evaluation of 1000 Genomes data by Castelli et al found the larger extent of genetic diversity of the *HLA-G* gene in African population when compared to European and Asian populations. While UTR-1 was substantially prevalent in European populations, it exhibited very low frequencies in African populations.[91] This is supported by the findings from Alvarez et al study which compared polymorphisms in exon 8 of *HLA-G* gene between Portuguese and people from Guinea-Bissau. In this study, Guinea-Bissau people displayed higher molecular diversity in exon 8 than Portuguese.[114]

Similarly, comparison of UTR haplotypes between the Malian population and Volunteers of Bone Marrow Donors (VBMDs) in France revealed a significantly lower frequency of UTR-1 in the Malian population. However, haplotypes UTR-2, UTR-3 and UTR-5 were more prevalent in the Malian population than in French VBMDs. Also, the frequencies of most SNPs in 3′UTR and 5′URR of *HLA-G* gene in the Malian population were significantly different from those displayed by French VBMDs population.[96]

A recent study by Sonon et al[99] genotyped the regulatory and coding regions of *HLA-G*, *-E* and *-F* genes among the Toffin population of Benin. The study identified 3, 4 and 2 new haplotypes in 5′URR, coding region and 3′UTR respectively. Interestingly, the study reports a relatively higher frequency of the null allele G*01:05N (11.41%) similar to that one reported by Matte et al among Shona women in Zimbabwe.[115] The findings support the idea that African populations are relatively more enriched with the null allele G∗01:05N compared to other populations in the world.[91] Furthermore, the findings from Julie et al reveals relatively higher frequencies of *HLA-G* alleles G∗01:04 and G∗01:03 in Teke Congolese and Tswa Pygmies than in south-eastern French population.[116]

Such uneven distribution of *HLA-G* alleles and haplotypes between populations may lead to different HLA-G expression profiles across different populations. Subsequently, populations with different HLA-G expression profiles may exhibit different clinicopathological characteristics of cancer with respect to HLA-G expression. It can theoretically be expected that populations laden with high expression of *HLA-G* haplotypes are more prone to develop cancer. The private, over-represented and under-represented haplotypes in the African populations may have an influence on HLA-G expression and modulating effect on different cancer susceptibility. It is therefore important to explore the oncological implications of these differing *HLA-G* genetic structures across populations by conducting more research in the African population.

Conclusions

Sufficient studies show that HLA-G is an immunosuppressive molecule involved in effecting the tumor cells immune escape and hence contributing to malignancy development. The expression of HLA-G can be influenced by the genetic configuration of its gene, as the gene bears some polymorphisms, especially in the 5′URR and 3′UTR regions affecting its expression. The polymorphisms in these regions are clustered into haplotypes which are unevenly distributed across different populations worldwide. Amid the existing controversies over how HLA-G relates to cancer, such uneven distribution of *HLA-G* haplotypes espouses the need to explore the oncological implication of HLA-G in the currently understudied African ethnicities. Such studies will broaden our understanding of HLA-G in relation to carcinogenesis. It is important to encourage further studies on tumorigenic influence of HLA-G due to its potential application in clinical settings, such as serving as a diagnostic and prognostic biomarker and being an immune checkpoint target for immunotherapy treatment of cancer.

Author contributions

IA reviewed the literature and developed the first draft of the manuscript. ND, TM, GA and IN reviewed the drafts under development and provided comments. FA, AA and ASN provided guidance on literature search and organization of manuscript, reviewed and approved the final manuscript.

Conflict of interests

The authors have no conflicts of this work.

Abbreviations

APCs Antigen-presenting cells
AU-ROC Area under receiver operating characteristic curve
CEA Carcinoembryonic
CA Cancer antigen
COV Cut-off value
DC Dendritic cell
HLA-G Human leukocyte antigen G
ILT Immunoglobulin-like transcript
ITIM Immuno-receptor tyrosine based inhibitory motif
KIR Killer cell immunoglobulin-like receptors
MHC Major histocompatibility complex
MDSCs Myeloid-derived suppressor cells
NK Natural killer
NSCLS Non-small cell lung cancer
SNPs Single nucleotide polymorphisms
UTR Untranslated region
VBMDs Volunteers of bone marrow donors

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