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Review

Human leukocyte antigen (HLA)-G and cervical cancer immunoediting: A candidate molecule for therapeutic intervention and prognostic biomarker?

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ABSTRACT

While persistent infection with oncogenic types of human *Papillomavirus* (HPV) is required for cervical epithelial cell transformation and cervical carcinogenesis, HPV infection alone is not sufficient to induce tumorigenesis. Only a minor fraction of HPV infections produce high-grade lesions and cervical cancer, suggesting complex host–virus interactions. Based on its pronounced immunoinhibitory properties, human leukocyte antigen (HLA)-G has been proposed as a possible prognostic biomarker and therapeutic target relevant in a wide variety of cancers and viral infections, but to date remains underexplored in cervical cancer. Given the possible influence of HLA-G on the clinical course of HPV infection, cervical lesions and cancer progression, a better understanding of HLA-G involvement in cervical carcinogenesis might contribute to two aspects of fundamental importance: 1. Characterization of a novel diagnostic/prognostic biomarker to identify cervical cancer and to monitor disease stage, critical for patient screening; 2. Identification of HLA-G-driven immune mechanisms involved in lesion development and cancer progression, leading to the development of strategies for modulating HLA-G expression for treatment purposes. Thus, this systematic review explores the potential involvement of HLA-G protein expression and polymorphisms in cervical carcinogenesis.

Contents

Abbreviations: 3′UTR, untranslated region; 5′URR, upstream regulatory region; ADC, cervical adenocarcinoma; AGUS, atypical glandular cells; APC, antigen presenting cells; ATG, start codon; Cd, cytoplasmatic domain; CD, cluster of differentiation; CIN, cervical intraepithelial neoplasia; CpGs, cytosine guanine dinucleotide; DC, dendritic cells; Del, deletion; ELISA, enzyme-linked immunosorbent assay; FasL, Fas, Fas ligand binding; FIGO, International Federation of Gynecology and Obstetrics; HC, heavy chain; HLA, human leucocyte antigen; HLA-G, human leucocyte antigen-G; HPV, human papillomavirus; HR, high-risk; HSIL, high grade SIL; Ia, classical HLA class I; Ib, non-classical HLA class I; IFN, interferon; IHC, immunohistochemistry; IL-10, interleukin-10; ILT, immunoglobulin-like transcripts; Ins, insertion; KIR, killer cell immunoglobulin-like receptors; KIR2DL4, KIR/CD158D; LILR, leukocyte immunoglobulin-like receptor; LR, low-risk; LSIL, low grade SIL; mAb, monoclonal antibody; MHC, major histocompatibility complex; mHLA-G, membrane-bound HLA-G; miRNA/miR, microRNA; mRNA, messenger ribonucleic acid; MSCs, mesenchymal stem cells; NK, natural killer; NR, not reported; PCR, polymerase chain reaction; qRT-PCR, quantitative real time-polymerase chain reaction; RT-PCR, real-time polymerase chain reaction; SCC, squamous cell cervical carcinoma; SCCW, SCC with lymph node metastasis; SCCWT, SCC without lymph node metastasis; sHLA-G, soluble HLA-G; SIL, squamous intraepithelial lesion; SNP, single nucleotide polymorphisms; SP, signal peptide; ST, stop; TAM, tumor associated macrophages; TGF, transforming growth factor beta; Th1, T helper-1; Th2, T helper-2; TM, transmembrane; TNF, tumor necrosis factor; Tregs, regulatory T cells; VEGF, vascular endothelial growth factor; WB, western blot; WOK, web of knowledge; α-HPV, alpha HPV species; β2-m, β2-microglobulin

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1. Introduction

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of female cancer mortality worldwide [1]. Persistent infection by oncogenic types of human *Papillomavirus* (HPV) is required for cervical epithelial cell transformation leading to cervical cancer [2]; however, HPV infection by itself is not sufficient to induce tumorigenesis. HPV infection is often transient because the host immune response can generally control viral invasion, leading to the regression of lesions [3]. Only a minor fraction of HPV infections produce high grade squamous intraepithelial lesions (HSIL) (10% of HPV infections) and cervical cancer (less than 1% of HPV infections) [4,5], suggesting that complex host–virus interactions determine HPV-induced cancer risk and lesion progression to cancer [6]. Therefore, a plethora of different mechanisms most likely contribute to HPV infection persistence and the progression of precancerous lesions to cervical cancer [4,5].

Several studies now support a critical role for immunosuppressive mechanisms in promoting HPV-induced cervical cancer, either by suppressing the capacity of the host to overcome HPV infection or by preventing the elimination of precancerous and HPV-transformed cells. Innate immunity is believed to be critical in controlling both HPV infections and HPV-associated cancers [7]. The host's genetic variations that impact the immune response likely determine those who are at higher risk for progression to cervical carcinoma among infected individuals [6]. The major histocompatibility complex (MHC), referred to as the human leucocyte antigen (HLA) system in humans, is involved in the identification of foreign antigens and activation of the immune system, and is therefore considered a probable participant in HPV and other viral infections [8–10]. Based on its pronounced immunoinhibitory properties, HLA-G has been suggested as a possible prognostic biomarker and therapeutic target relevant to a wide variety of cancers [11–13] and viral infections [14,15]. However, the possibility that HLA-G gene polymorphisms and/or protein expression affects HPV-infection persistency and cervical cancer risk remains underexplored.

1.1. HLA-G structure and function

HLA-G is a unique, non-classical HLA class I (Ib) molecule involved in various immunosuppression mechanisms. Although the HLA-G gene shares several similar characteristics with classical HLA class I (Ia) molecules, its expression pattern, peptide binding properties, restrictive tissue distribution, low coding region polymorphisms, and inhibitory action on immune cells are distinct [16–18]. Importantly, at least seven splicing variants of HLA-G primary messenger ribonucleic acid (mRNA) transcript have been described. Surprisingly, the expression of HLA Ia molecules (HLA-A, -B, and -C) are typically downregulated in tumor cells, suggesting there are different expression patterns for the various classes of HLA molecules in the development of human cancers. HLA molecules potentially contribute to a tumor-associated immunosuppressive phenotype [17,19].

The HLA system is a 3.6 Mb high-density gene region located at the 6p21.3 chromosome that contains more than 200 genes [20,21] (see Fig. 1A). The HLA-G gene is composed of eight exons and seven introns. In contrast to HLA Ia loci, HLA-G has a stop codon at exon 6, leading to a short cytoplasmic tail. The HLA-G gene also includes a 5′ upstream regulatory (or promoter) region (5′URR) extending at least 1.4 kb from the initial ATG start codon [22] as well as an extended 3′ untranslated region (3′UTR) [23] (see Fig. 1B). The alternative splicing of the HLA-G primary transcript results in seven different isoforms; four membrane-bound HLA-G (mHLA-G1, -G2, -G3 and -G4) and three soluble (sHLA-G5, -G6 and -G7) proteins isoforms [24,25]. HLA-G1 is the full-length HLA-G molecule, HLA-G2 lacks exon 3, HLA-G3 lacks exons 3 and 4, and HLA-G4 lacks exon 4. HLA-G1 to -G4 are membrane-bound molecules due to the presence of the transmembrane and cytoplasmic tail encoded by exons 5 and 6. HLA-G5 is similar to HLA-G1 but retains intron 4, HLA-G6 lacks exon 3 but retains intron 4, and HLA-G7 lacks exon 3 but retains intron 2. HLA-G5 and -G6 are soluble forms due to the presence of intron 4, which contains a premature stop codon to prevent the translation of the transmembrane and cytoplasmic tail. HLA-G7 is soluble due to the presence of intron 2, which contains a premature stop codon [22–25] (see Fig. 1C).

The alternate splicing of the primary HLA-G transcript exemplifies a key aspect of its regulation and indicates that the amount and type of HLA-G expression may be cell type dependent. To date, most available information is known about the HLA-G1 molecule and its soluble counterpart HLA-G5. These molecules are composed of the heavy chain (HC), consisting of three globular domains (α1, α2, α3) non-covalently bound to β2-microglobulin (β2-m). In contrast, the other isoforms lacking one or two globular domains are smaller and cannot bind β2-m or present peptides [26,27] (see Fig. 1A, B and C).

In healthy individuals, basal levels of HLA-G gene transcription are observed in most cells and tissues. However, translation of the HLA-G gene transcript into protein is restricted to trophoblasts at the fetal-maternal interface [28] and to thymic epithelial, corneal, mesenchymal stem cells (MSCs), nail matrix, pancreatic cells, and erythroid and endothelial precursors in adults [29]. HLA-G expression can also be postnatal in pathological conditions, including malignant transformed tissue [27,30], infectious diseases [31], inflammatory and autoimmune diseases [11], and allogeneic transplantation [11,13,18].

HLA-G protein expression broadly affects many aspects of human innate and adaptive immunity [18,26] and inhibits cell-mediated immunity through interactions with the receptors expressed on lymphoid, myeloid, and natural killer (NK) cells [22,32–34]. The selective induction of HLA-G molecules might enable viruses and cancer cells to bypass host immunosurveillance and elimination mechanisms [26,27,35]. HLA-G is involved in suppression of cytotoxic activity of T and NK cells, cluster of differentiation protein 4 (CD4+) T cell alloproliferative responses, T and NK cell proliferation, and maturation of antigen presenting cells (APCs) via direct binding to inhibitory receptors expressed on the surface of various immune cells. In addition, sHLA-G forms are involved in the induction of suppressive regulatory T cells, activated CD protein 8 (CD8+) T and NK cell apoptosis, and the upregulation of inhibitory receptors [29] (see Fig. 2).

Collectively, studies have indicated that HLA-G inhibits the function of major immune cells through interaction with their inhibitory receptors [22,35]. Three HLA-G-recognized killer cell immunoglobulin-like

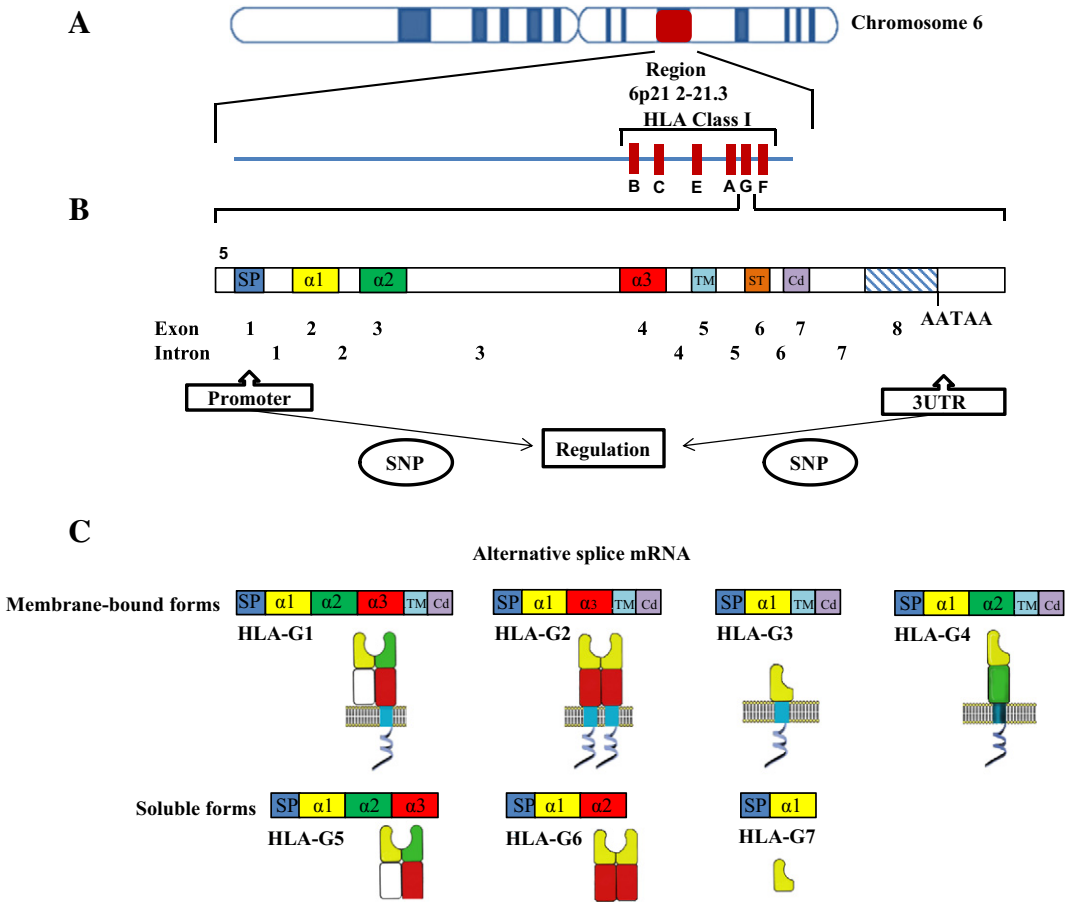

Fig. 1. The human leukocyte antigen (HLA)-G gene, transcription, and isoforms. A) HLA-G gene location in chromosome 6 [16–18,20,21]; B) the HLA-G gene structure consists of 7 introns (white color) and 8 exons (different colors). The HLA-G gene promoter contains regulatory elements to regulate HLA-G gene transcription. The 3′UTR of the HLA-G gene also contains several regulatory elements (SP, signal peptide; TM, transmembrane; ST, stop; Cd, cytoplasmic domain; SNP, single- nucleotide polymorphisms [22–27]; C) the HLA-G primary transcript can be spliced into 7 alternative messenger ribonucleic acid (mRNA) denoted as HLA-G1 to -G7. The HLA-G protein exhibits a heterodimer structure consisting of globular domains (α1, α2, α3, TM, and Cd domains) and a light chain (β2-microglobulin) [24–27].

receptors (KIRs) have been identified including immunoglobulin-like transcripts (ILT)2/CD85j/(leukocyte immunoglobulin-like receptors-LILR)B1, ILT4/CD85d/LILRB2, and KIR2DL4/CD158d. In addition to their expression on NK cells (KIR2DL4 and ILT2), these receptors have been detected on all T and B cells (ILT2), monocytes/macrophages (ILT2 and ILT4), and dendritic cells (DC) (ILT2 and ILT4). Although the ILT2 and ILT4 KIRs can interact with other HLA I ligands, they show the highest binding affinity to HLA-G [22,32–36]. KIR2DL4, on the other hand, is expressed by all NK cells and is thought to be an HLA-G specific receptor.

The HLA-G and effector immune cell interactions are accompanied by the onset and maintenance of tolerance at different stages of the immune response including recognition, differentiation, proliferation, cell lysis and cytokine secretion [27,37–43]. Consistent with its immunosuppressive function, HLA-G expression is associated with better patient outcomes after kidney, liver [44,45] and heart transplantation [38]. However, HLA-G expression in various tumors is related to poor patient prognosis, which is primarily attributed to immune evasion by HLA-G-overexpressing tumors [17,32,46] (see Fig. 2).

1.2. HLA-G and cancer immunoediting

Cancer is essentially considered a complex cellular disease caused by abnormalities in the genome, metabolome and interactome of transformed cells. However, cancer development is actually a multi-step progressive process that involves a sequence of biological dysfunctions in multiple systems ultimately allowing sustained proliferative and insensitivity to antiproliferative signaling. Also critical for the establishment and progression of tumors is the capacity to evade immune elimination. The immune system can specifically identify and eliminate tumor cells based on the expression of tumor-specific antigens or molecules induced during malignant cell transformation [47,48]. This process is referred to as tumor immune surveillance [26]. The concept that the immune system can scan the body for microbial pathogens and aberrant cancer cells and subsequently employ innate and adaptive immune responses to eliminate them was theorized more than 100 years ago by Paul Ehrlich [49].

Despite the process of immune surveillance, tumors can still develop in the presence of a functioning immune system [48,50]. This occurs through tumor immunoediting, a process comprised of three major phases: elimination, equilibrium and escape [51]. The elimination phase corresponds to cancer immunosurveillance and engages cells from the innate and adaptive immune responses that recognize and eliminate tumor cells. If only partial eradication of tumor cells occurs, an equilibrium between the tumor and the immune system develops that leads to the production of less immunogenic tumor cells by clonal selection (equilibrium phase). Finally, these tumor cell variants escape any antitumour responses, thereby sustaining growth and progression (escape phase) [51]. HLA-G is involved in every phase of tumor immunoediting, exemplifying one of the immunosuppressive strategies employed by various tumors to evade the immune response [26,51,52].

HLA-G performs its immune suppressive activity in several ways. HLA-G can decrease the elimination of tumor cells by inhibiting the

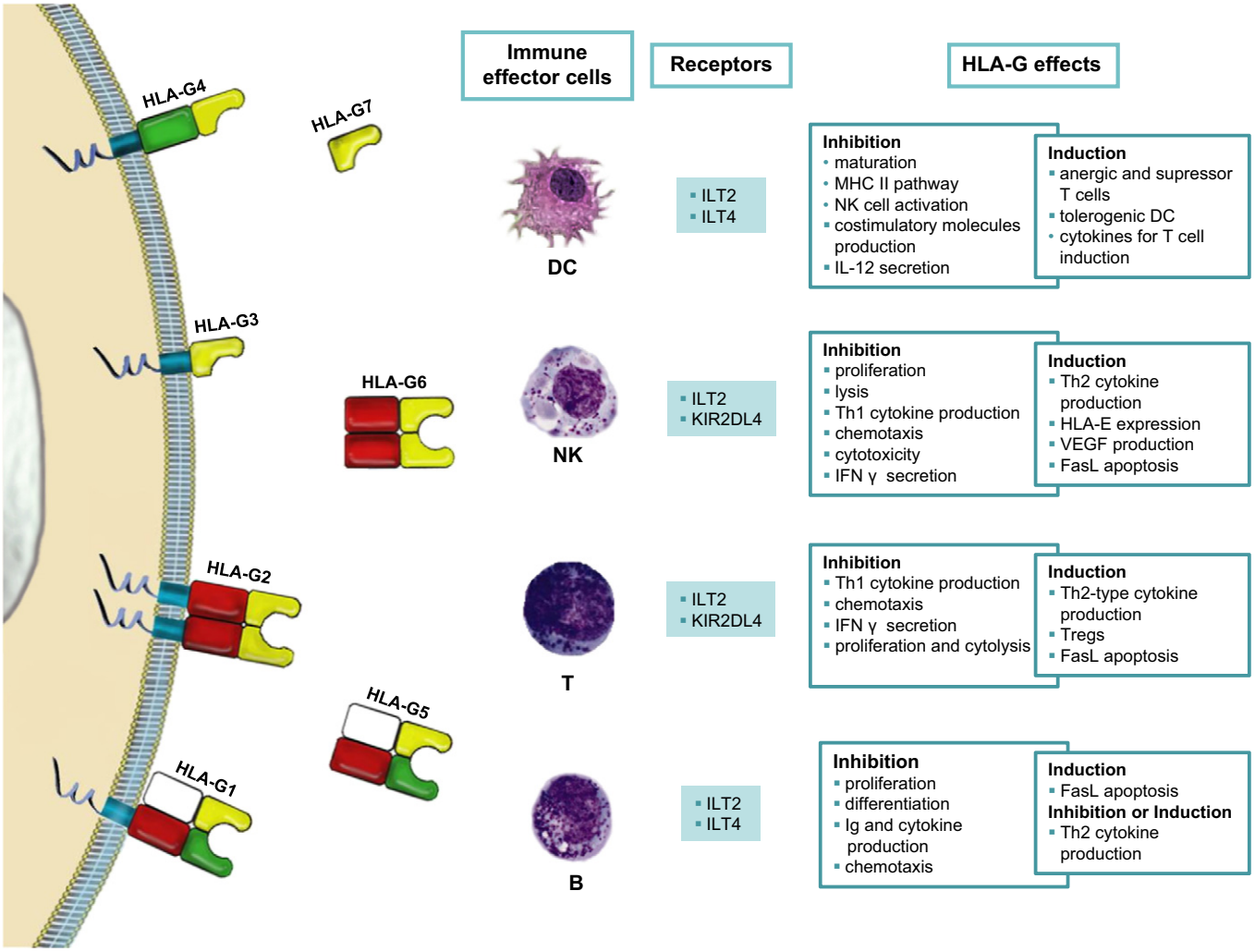

Fig. 2. Schematic representation of how tumor cells downregulate immunosurveillance by expressing HLA-G. Soluble (s) and membrane-bound (m) HLA-G exerts negative immunoregulatory functions by interacting with immunoglobulin-like receptors (KIRs) such as immunoglobulin-like transcripts (ILT)2/CD85j/(leukocyte immunoglobulin-like receptors-LILR)B1, ILT4/CD85d/LILRB2, and KIR2DL4/CD158d. In addition, to their expression on natural killer (NK) cells (KIR2DL4 and ILT-2), these receptors have also been detected on all T and B cells (ILT-2), monocytes/macrophages (ILT-2 and ILT-4), and dendritic cells (DC) (ILT-2 and ILT-4). KIR2DL4 is expressed by all NK cells and is thought to be a HLA-G specific receptor [22,32–36]. MHC, major histocompatibility complex; Th1, T helper 1 cytokine; IFN, interferon; VEGF, vascular endothelial growth factor; FasL, ligand type-II transmembrane protein to the tumor necrosis factor (TNF) family; Tregs, regulatory T cells.

cytotoxic function of T and NK cells via trogocytosis (i.e. intercell transference of viable HLA-G molecules), which renders competent cytotoxic cells unresponsive to tumor antigens [48,50]. In this context, structural and functional alterations of the HLA Ia antigens occur frequently in cancer and serve to circumvent antigen-specific T-cell responses [19,53]. Cells lacking HLA Ia molecules are more susceptible to elimination by NK cells. When released from the inhibitory effect of HLA molecules on their KIR receptor, activated NK cells can eliminate HLA Ia negative tumor cells [27,47]. However, such elimination may not always be effective and the tumor cells expressing HLA-G can evade elimination. The overall functional relevance of HLA-G varies according to its expression by tumor cells or tumor-infiltrating cells [54].

Additionally, HLA-G can perform its immune suppressive activity by indirect mechanisms. One such possibility is the expression of HLA-E, another type of HLA Ib molecule, which is expressed on tumor-associated macrophages (TAMs) that can directly bind peptides derived from HLA-G. This molecule can interact with the NK cell inhibitory receptor (CD94/NKG2A) resulting in the production of immune suppressive cytokines, interleukin-10 (IL-10) and transforming growth factor beta (TGF-ß) by NK cells, thus interfering with the activation of T cells, DC and APCs in general [27] (see Fig. 3).

1.3. HLA-G expression and polymorphisms in tumors

1.3.1. HLA-G expression in tumors

In 1998, Paul et al. provided the first evidence that functional HLA-G protein expression protects melanoma cell lines from NK-mediated cell death, potentially enhancing escape from the host's immune response [55]. Further studies over the following decade have now demonstrated that HLA-G protein expression is evident in at least 16 tumors of ectodermic, mesodermic, or endodermic origin [56,57]. Overall, HLA-G has been shown to be expressed in primary solid tumors and metastases including breast and ovarian malignancies [58–60], leukemia, lymphomas, and myeloma [61–64]. HLA-G can be expressed in malignant effusions [58–71] as peritoneal and pleural effusions in mesothelioma [70] as well as in exudates from other cancer patients [7]. HLA-G can also be found on tumor-infiltrating cells, particularly lymphocytes infiltrating cervical cancers [65], macrophages in hydatidiform moles [66], activated microglia/macrophages in glioblastomas [67], myelo-monocytic cells in lung pathology [68], CD8 + T cells in breast cancer [54], and macrophages and DCs in lung cancer [69].

In summary, the clinical relevance of HLA-G in cancer is supported by the following observations: 1. HLA-G protein expression is

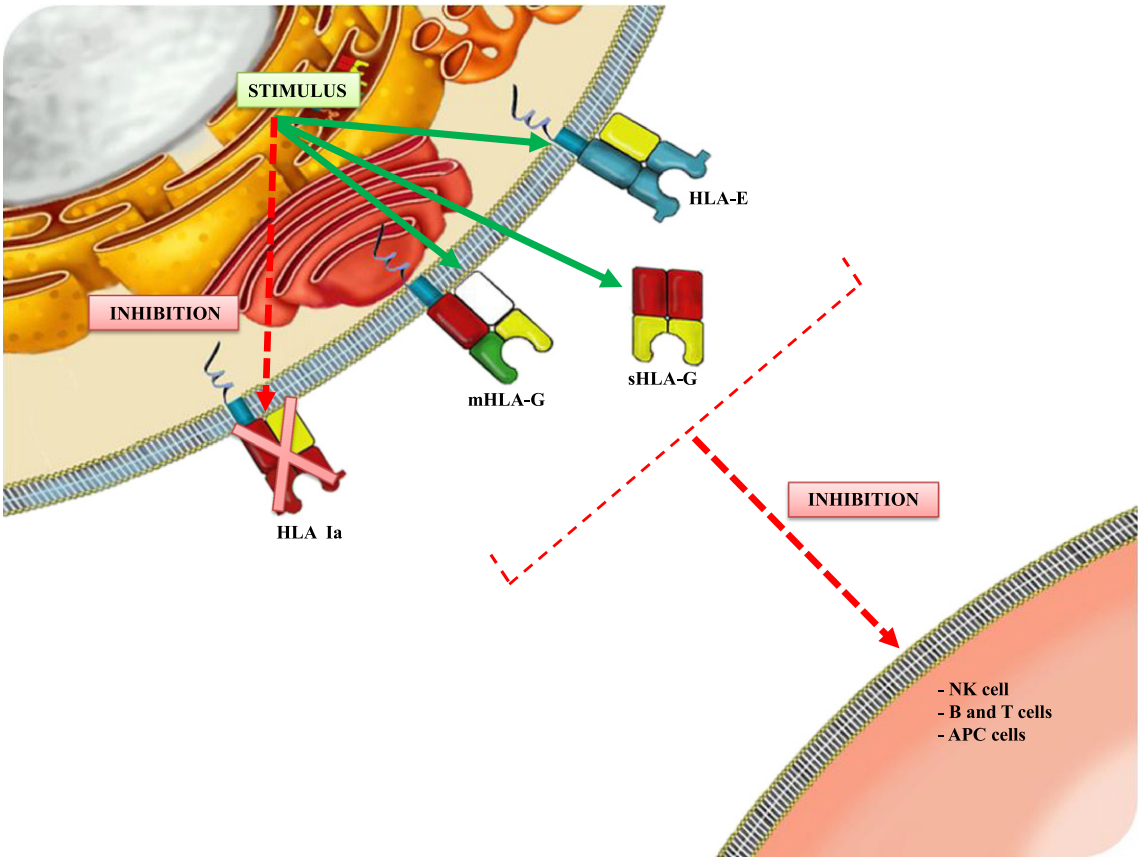

Fig. 3. Schematic representation of an alternative immunosuppressive mechanism mediated by HLA-G secreted by tumor cells or antigen activated tumor-associated cells. Structural and functional alterations of the HLA Ia antigens occur frequently in cancer and serve to circumvent antigen-specific T-cell response. Cells lacking HLA Ia are more susceptible to elimination by NK cells but their elimination may be hampered by tumor cells expressing HLA-G. Membrane-bound HLA-G (mHLA-G) and soluble (sHLA-G) proteins inhibit NK, B, and T cells as well as antigen presenting cells (APC). HLA-G also promotes the expression of HLA-E, a HLA Ib molecule that inhibits NK and T cell reactivity [19,26,27,47–51].

heterogeneous among various types of tumors but higher in tumor tissues with more infrequent localization to the adjacent normal tissue, suggesting a specific association between HLA-G expression and malignant transformation/progression [51,58–64]. 2. HLA-G is expressed in solid tumors of high histological grades and advanced clinical stages suggesting a specific association between HLA-G expression and malignant progression [58–74]. 3. The use of HLA-G as a prognostic marker has been proposed since HLA-G expression in biopsies and/or high levels of sHLA-G in plasma from patients have been significantly correlated with poor prognosis [61,72–77]. These data highlight a significant role for HLA-G in the immune surveillance of solid tumors and progression of neoplastic disease [26–29,31–38,44–46,78–82].

1.3.2. HLA-G sequence polymorphisms in tumors

Limited HLA-G coding region variability has been observed in worldwide populations [83]. Based on the gametic phase (haplotypes) of 73 single-nucleotide polymorphisms (SNPs) observed between exon 1 and intron 6, 50 alleles and 16 proteins have been described for HLA-G (IMGT HLA database, April 2013) [84].

Unlike the polymorphisms in HLA Ia molecules that are mainly concentrated around the peptide-binding groove, the HLA-G polymorphisms are distributed between the α1, α2, and α3 domains [23]. To date, SNPs have been described in the 5′URR, exon 2, exon 3, and the 3′UTR of exon 8 of the HLA-G gene [27] (see Fig. 4). These polymorphisms alter the affinity of gene-targeted sequences for transcriptional or post-transcriptional factors [27]. In particular, rs66554220, a 14-bp Insertion/Deletion (*Ins*/*Del*) polymorphism in the 3′UTR of the HLA-G gene, is associated with HLA-G mRNA stability and splicing; *Ins* and *Del* alleles are associated with decreased or increased mRNA stability, respectively [85]. Transcripts from the 14-bp *Ins* allele can undergo an additional splicing step that removes 92 bases, including the region where the 14-bp *Del* is located, yielding more stable transcripts in vitro [86]. However, in vivo studies revealed that 14-bp *Ins* allele exhibits decreased HLA-G expression [87,88]. The *Ins*/*Del* of the 14-bp also alters the set of microRNAs (miRNAs or miRs) that are capable of binding the locus [89], thus influencing RNA turnover and miRNA-mediated repression of translation. The C/G SNP located less than 200-bp away from the 14-bp polymorphic site at position rs1063320 is also thought to influence miRNA binding. At this polymorphic site, the G allele favors the targeting of three miRNAs (miR-148a, -148b and -152) to the binding site reducing HLA-G expression. Both of these described polymorphisms are in linkage disequilibrium [90,91]. Thus, polymorphic sites present in the coding and non-coding regions of the HLA-G gene may potentially affect its function and expression [6,23].

Previous studies have linked HLA-G polymorphisms with certain diseases, such as autoimmune diseases, preeclampsia, transplantation, and neoplasias [91,92] as well as the clinical course of these diseases [27]. However, to date, limited information has been published on the association of HLA-G polymorphisms in tumor cells with the level of HLA-G expression and/or clinical outcome of patients [27].

Despite the requirement for studies to clarify the role of the identified HLA-G gene SNP relative to HLA-G expression, the 14-bp polymorphism has been shown to account for differences in HLA-G mRNA and protein expression profiles [27]. Reduced HLA-G transcript levels were observed in HLA-G genotypes with a 14-bp *Del*, whereas reduced sHLA-G serum and plasma levels and differences in the alternative splicing of HLA-G transcripts and HLA-G mRNA stability were detected in 14-bp *Ins*/*Ins* HLA-G genotypes [89,92–94]. The *Del*/*Del* genotype is a recognized risk factor for viral infection [15] and tumor progression [95].

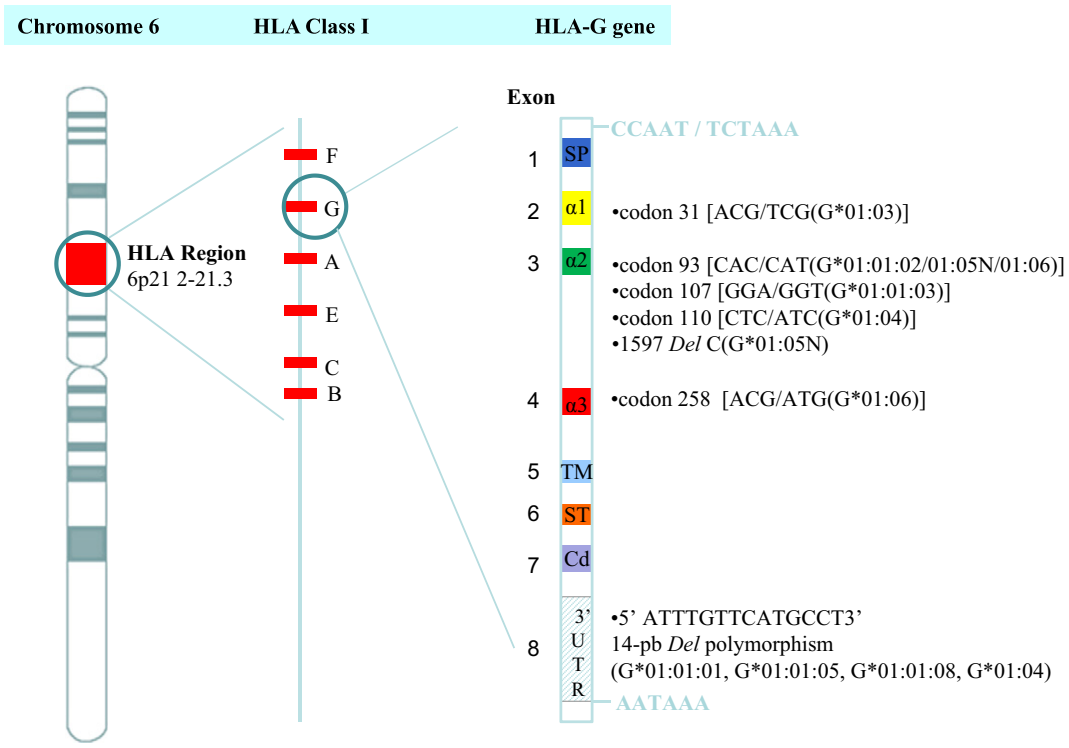

Fig. 4. HLA-G gene organization and polymorphisms. Some of the possible HLA-G gene polymorphisms described in the text are shown [6,23,27,83–89]. SP, signal peptide; TM, transmembrane; ST, stop; Cd, cytoplasmic domain.

2. Aim

Given the possible influence of HLA-G on the clinical course of HPV infection, cervical lesions and cancer, a better understanding of the involvement of HLA-G in cervical carcinogenesis may contribute to two fundamentally important aspects: 1. The characterization of a novel diagnostic/prognostic biomarker to identify cervical cancer and to monitor disease stage, which are critical for patient screening; 2. The identification of HLA-G-driven immune mechanisms involved in lesion development and progression to cancer which may lead to the development of strategies to modulate HLA-G expression for treatment purposes. Thus, this systematic review explores the potential involvement of HLA-G protein expression and polymorphisms in cervical carcinogenesis.

3. Methodology and delimitations

We performed a systematic review to identify studies focused on "HLA-G" and "cervical carcinogenesis" in PubMed, Embase and Web of Knowledge (WOK) databases for publications dating between January 1993 and April 2014 based on the PRISMA statement [96]. To identify original publications in the English language, researchers (ALPA, FG, JJVT, RPS, MWP) performed independent searches using various combinations of descriptors in PubMed/Embase or as a topic in WOK ("HLA-G antigens" and "uterine cervical neoplasms" or "uterine cervical dysplasia" or "papillomavirus infections" or "cervical intraepithelial neoplasia" or "squamous cell cervical carcinoma" or "human papillomavirus DNA tests" or "human papillomavirus 16" or "human papillomavirus 18" or "papillomaviridae" or "HPV genotype"). Abstracts were carefully selected to ensure publication originality and quantitative and qualitative consensus. Studies initially selected had to fit the following three criteria: the first criteria included original epidemiological and clinical studies involving humans. The second criteria was to exclude duplicate studies, review studies, case studies, reviews, comparative studies and letters to editor. The third criteria involved screening publications for eligibility based on use of molecular methods for the identification of HPV, and immunological or molecular methods for HLA-G alleles and expression. After consensus, the papers most closely related to the theme descriptors were selected for the study. Articles were then randomly distributed to investigators who acted as independent judges (ALPA, FG, JJVT, RPS, VRSS, CGB, SDB, SSM-E, MGB, MELC) to debate the inclusion of the paper in the final cohort for data extraction. To increase the sensitivity of the search, the references of the original articles were carefully reviewed for recovery articles that could be additionally utilized in this review. To ensure that all relevant data from each paper were included in the review, a final consensus was achieved following an additional examination of the full texts by two individual experts (MELC, SDB). In total, sixteen studies met our inclusion criteria (Fig. 5).

In total, sixteen studies met our inclusion criteria. These studies fell into two broad categories: HLA-G expression (n = 8) and HLA-G polymorphisms (n = 8) (Tables 1 and 2).

4. HLA-G expression in cervical carcinogenesis

Among the eight included studies evaluating the relationship between HLA-G expression and cervical carcinogenesis, six analyzed the expression of HLA-G in cervical tissues by immunohistochemistry, one analyzed tissue expression by immunohistochemistry and circulating sHLA-G, and one assessed HLA-G mRNA expression by real-time polymerase chain reaction (RT-PCR). The studies are summarized in Table 1.

The only one study analyzing HLA-G and IL-10 mRNA expression in cervical carcinogenesis was conducted by Yoon et al. Cervical tissues from Korean women were analyzed (40 squamous cell cervical carcinoma-SCCs and 15 normal tissues). Both HLA-G and IL-10 mRNA expression in SCC were significantly higher than in controls. A similar trend was seen for HLA-G and IL-10 protein expression. HLA-G mRNA expression was significantly correlated with HLA-G protein expression and a similar pattern was observed for IL-10; however statistical significance was not achieved. According to this study, no significant

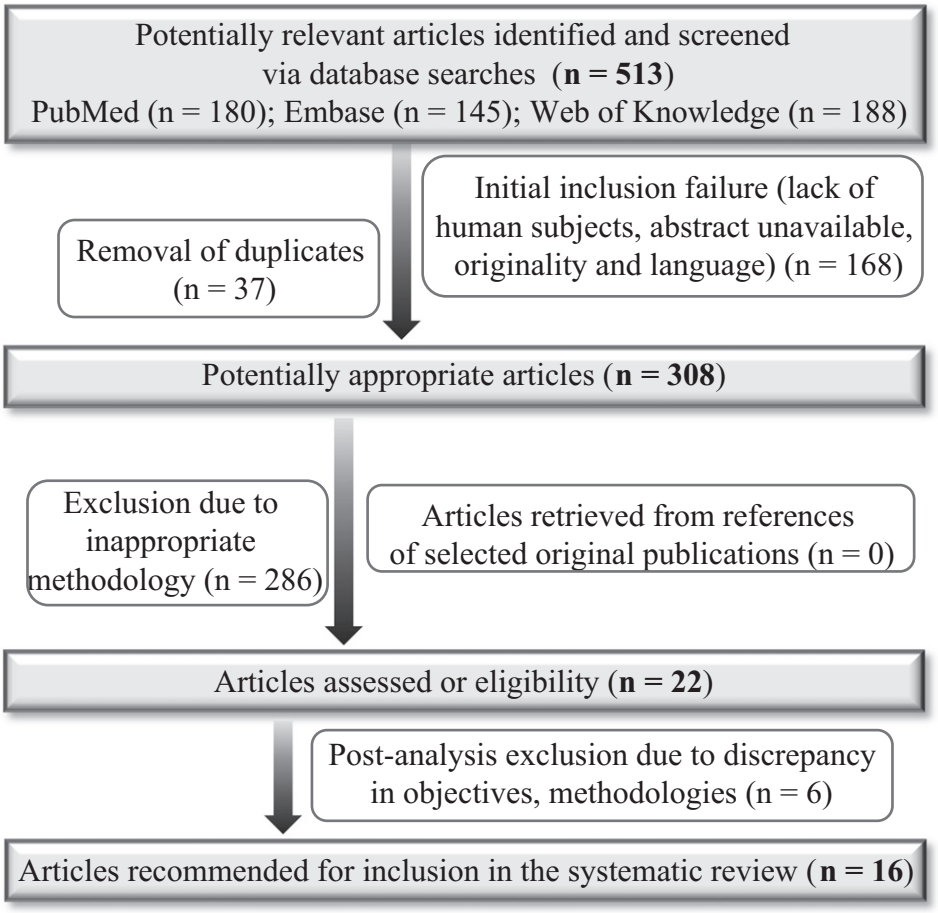

Fig. 5. Flow diagram of criteria to be met for inclusion in systematic review.

correlation was observed between HLA-G and IL-10 expression levels both at the mRNA and protein levels. An inverse relationship between FIGO (International Federation of Gynecology and Obstetrics) SCC stage and HLA-G mRNA expression was observed, with HLA-G mRNA expression levels significantly higher in early stage patients compared to advanced stage patients. The authors concluded that HLA-G and IL-10 might play an important role in SCC progression. Additionally, the high HLA-G mRNA expression levels associated with early stage SCC supports a possible role for HLA-G in early carcinogenesis [97]. However, it is important to emphasize that this study did not evaluate the expression of HLA-G in pre-invasive stages (cervical intraepithelial neoplasia-CIN), and therefore does not constitute a complete analysis of cervical disease progression from the earliest stages.

The first three studies published analyzing HLA-G expression by immunohistochemistry (IHC) reported no association between HLA-G overexpression and cervical carcinogenesis. Zhou et al. analyzed HLA-G expression using anti-HLA-G monoclonal antibody (mAb) 4H84 in CIN and SCC samples from Chinese women (19 normal tissues, 15 CIN I, 22 CIN II, 23 CIN III, and 34 SCC). A strong and uniform HLA-G expression was observed in normal epithelium (squamous and columnar), while only a small proportion of CINs and SCC samples exhibited significantly reduced expression of HLA-G. Additionally, HLA-G expression was not associated with any clinicopathological parameters [98].

Gonçalves et al. reported similar results after analyzing HLA-G expression by IHC with anti-HLA-G mAb 4H84 in cervical tissues from Brazilian women (10 normal tissues, 31 CIN I, 19 CIN II/III, 10 SCC, and 4 cervical adenocarcinoma-ADC). HLA-G was not expressed in any specimen presenting with CIN I-III or SCC. Conversely, HLA-E expression increased from CIN I to SCC. In addition, in areas exhibiting atypical glandular cells (AGUS), strong HLA-G staining was observed, whereas normal columnar cells were negative for HLA-G expression. The authors hypothesized that HLA-E rather than HLA-G may protect HPV-infected cells from immune surveillance given that HLA-G signal peptides are required for the cell-surface expression of the HLA-E molecule [99]. Moreover, the small sample size of SCC and ADC tissues examined in this study may have influenced the results.

Guimarães et al. also reported no relationship between HLA-G5 isoform expression and cervical carcinogenesis. Tissue samples from Brazilian women stratified according to the presence (n = 27) or absence (n = 52) of lymph node metastasis were analyzed by IHC for HLA-G5 expression using the specific anti-HLA-G mAb 5A6G7. Normal cervical specimens and negative controls did not present any squamous cells with positive immunostaining. HLA-G5 expression was low and similar in both groups (32.7% without metastasis and 29.6% with metastasis), encompassing a total of 25 cases (31.6%). HPV was detected in 74 cases (93.7%), with low HLA-G5 expression observed in all HPV-related cases. In the quantitative analysis of the metastasis group, HLA-G5 expression was significantly correlated with the corresponding lymph node metastatic cells. These authors argued that it remains to be determined why some SCC patients express HLA-G5, whereas others do not [100]. This study also did not evaluate the expression of HLA-G in pre-invasive disease stages (CIN) and therefore results may have been biased.

On the other hand, despite the fact that the three studies described above failed to demonstrate a direct association between HLA-G overexpression and cervical carcinogenesis, more recent articles (published from 2010 and on), presented different results.

Dong et al. studied HLA-G expression using anti-HLA-G mAb HGY in Chinese women (16 CIN I, 30 CIN II, 9 CIN III, and 116 SCC) with or without HPV infection. HLA-G expression increased from CIN I to CIN II/III

Table 1
Human leukocyte antigen-G expression in cervical carcinogenesis.

First author/year	Country	Period of study	Sample size/number	Age (years): mean ± SD, range	Laboratorial method	Brief conclusion	Reference
Zhou/2006	China	2002–5	CIN I: 15 CIN II: 22 CIN III: 23 SCC: 34 Normal: 19	CIN I: 26–64 CIN II: 30–52 CIN III: 30–71 SCC: 26–71 Normal: 36–68	IHC/mAb 4H84	CIN I–III and SCC showed significantly reduced expression of HLA-G.	[98]
Yoon/2007	Korea	2004–5	SCC: 40 Normal:15	SCC: 51, 37–69 Normal: NR	qRT-PCR/WB	HLA-G mRNA expression was associated with early-stage SCC	[97]
Gonçalves/2008	Brazil	NR	CIN I: 31 CIN II/III: 19 SCC: 10 ADC: 4 Normal: 10	NR	IHC/mAb 4H84	HLA-G staining was not observed in any cervical lesions (CIN I–III) or ISCC.	[99]
Guimarães/2010	Brazil	1994–2004	SCCWT: 52 SCCW: 27	SCCWT: 19 ± 1.5 SCCW: 17 ± 0.7	IHC/mAb 5A6G7	HLA-G5 expression was low and similar in both groups (32.7% SCCWT and 29.6% SCCW) with low expression in all HPV-related cases.	[100]
Dong/2010	China	2002–8	CIN I: 16 CIN II: 30 CIN III: 9 SCC: 116	CIN I: 32, 24–42 CIN II: 33, 19–68 CIN III: 35, 27–46 SCC: 44, 27–84	IHC/mAb HGY	HLA-G expression was associated with cervical disease progression and HPV-16/-18 infection	[65]
Zheng/2011	China	2008–9	CIN I: 15 CIN II: 28 CIN III: 36 SCC: 40 Normal: 22	CIN/SCC: 44, 24–75 Normal: 40,20–73	IHC/mAb 4H84 Elisa	HLA-G IHC expression and sHLA-G levels were increased in CIN and SCC patients.	[101]
Li/2012	China	2005–10	CIN III: 14 SCC: 129 Normal: 32	CIN III: 50, 29–74 SCC: 48, 22–86 Normal: 45, 22–82	IHC/mAb 4H84	HLA-G expression increased from CIN III to SCC and was associated with disease progression.	[102]
Rodríguez/2012	Colombia	2004–5	CIN III: 9 SCC: 54	43.1 ± 10.8, 25–63	IHC/mAb 4H84	HLA-G expression increased from CIN III to early-stage SCC; however, a reduction was observed in advanced SCC stages.	[103]

Abbreviations: ADC = cervical adenocarcinoma, CIN = cervical intraepithelial neoplasia, Elisa = enzyme-linked immunosorbent assay, HPV = human *Papillomavirus*, IHC = immunohistochemistry, mRNA = messenger ribonucleic acid, NR = not reported, qRT-PCR = quantitative real time-polymerase chain reaction, SCC = invasive squamous cell cervical carcinoma, SCCWT = SCC without lymph node metastasis, SCCW = SCC with lymph node metastasis, sHLA-G = soluble HLA-G, WB = western blot.

and was highest in patients with SCC. HLA-G expression was also significantly higher in CIN samples positive for HPV-16/-18 than CIN negative for HPV. The authors concluded that HLA-G expression is associated not only with disease progression but also with HPV infection [65].

In 2011, Zheng et al. examined the HLA-G expression levels with anti-HLA-G mAb 4H84 in 119 Chinese women (22 adjacent tumor-negative cervical tissues, 15 CIN I, 28 CIN II, 36 CIN III, and 40 SCC). HLA-G expression was negative in normal tissues, thus the expression of HLA-G in CIN and SCC (45% of samples) was significantly increased compared with adjacent normal tissues. This study also analyzed clinicopathological parameters, demonstrating significant correlations between HLA-G expression and the size of the main lesion, parametrial invasion, and lymph node metastasis. Additionally, sHLA-G expression in the plasma (172 CIN and SCC, and 20 healthy controls) was investigated. sHLA-G levels were significantly increased in CIN II, CIN III, and SCC groups compared to normal and CIN I groups, with no difference detected among the normal and CIN I groups. Upon examination of clinicopathological parameters, sHLA-G levels were found to be significantly associated with differentiation, lymph node metastasis, and parametrial invasion. The sensitivity and specificity of sHLA-G for SCC detection were 73.30% and 65.71%, respectively, suggesting that sHLA-G detection in plasma may have significance in the early detection of SCC [101]. It is noteworthy that this was the only paper found by us in the literature that studied sHLA-G expression in cervical carcinogenesis.

In 2012, two independent studies also showed a positive correlation between HLA-G expression and cervical carcinogenesis. Li et al. analyzed HLA-G expression in samples from Chinese women (32 normal adjacent tumor cervical tissues, 14 CIN III, and 129 SCC) by IHC with the anti-HLA-G mAb 4H84. HLA-G expression was absent in normal tissues, with expression increasing from CIN III (35.7%) to SCC (62.8%). Among the SCC cases, HLA-G expression in FIGO stages I, II, and III + IV was 53.6%, 76.3%, and 100.0%, respectively. Thus, HLA-G expression was associated with disease progression in patients with CIN III and SCC [102]. It should be noted that CIN I and CIN II samples were not included in this study and CIN III cases were limited.

The second study published in 2012 was performed by Rodriguez et al. and analyzed HLA-G (with anti-HLA-G mAb 4H84), HLA Ia, and IL-10 expression in samples from Colombian women (9 CIN III and 54 SCC). Absent or weak HLA Ia expression was observed in 85% of cases. IL-10 was expressed in 46.6% of cases while HLA-G was expressed in 27.6% of cases. Moreover, the majority of HLA-G positive cases (87.5%) exhibited upregulation of the IL-10 cytokine. Most IL-10-positive cases were associated with HLA Ia downregulation and significantly increased HLA-G expression was noted in patients with HLA Ia downregulation compared to those with normal HLA Ia. Finally, HLA-G upregulation from CIN III to early stages of SCC was noted, however a significant association with the more advanced stages of SCC was not observed. Statistically significant differences in survival among women expressing various levels of HLA-G were not found. Taken together, these results suggest that IL-10 secretion in the SCC microenvironment may promote local immunosuppression by upregulating HLA-G expression and downregulating HLA Ia expression, which in turn can promote a lower susceptibility to specific NK cell and cytotoxic T lymphocyte-mediated killing [103]. As noted in previous papers already discussed above, the results of this study may have been influenced by the lack of CIN I and CIN II samples as well as limited cases of CIN III.

In summary, HLA-G expression in SCC tissue has been found to be increased [65,95,101–103]; reduced [98] or absent/weak [99,100] in comparison to HPV-induced lesions at an earlier stage according to data currently available. Discrepancy in various studies examining HLA-G expression levels in tissue staining may have also been a result of different HLA-G antibodies used, suggesting the need to possibly standardize antibodies used for screening and/or staging purposes. Consequently, there is still a need for further studies to establish whether

Table 2
Human leukocyte antigen-G sequence polymorphism and cervical carcinogenesis.

First author/year	Country	Period of study	Sample size	Age (years): mean ± SD, range	Laboratorial method	Polymorphism and protection	Polymorphism and susceptibility	Brief conclusions	Reference
Simões/2009	Brazil	NR	LSIL: 68 HSIL: 57 Normal: 94	LSIL/HSIL: 31.1 ± 10.9 Normal: 34.1 ± 15.1	PCR	HLA-G*01:03 allele and SIL HLA-G*01:01/G*01:04 genotype and HSIL	HLA-G*01:04/14-bp *Ins*/*Del* and SIL HLA-G*01:04/14-bp *Ins*, HPV-16/-18 co-infections and HSIL	HLA-G polymorphism may be associated with HPV infection and SIL, possible profile of predisposition to SCC	[104]
Ferguson/2011	Canadian	1996–2001	636	21, 17–42	PCR	None detected	HLA-G*01:01:02 and HLA-G*01:01:08 alleles and HPV-16 and genotypes from alpha species 1, 8, 10, and 13 HLA-G*01:01:02, HLA-G*01:03 alleles and persistence of HPV-16 and genotypes from alpha species 2, 3, 4, and 15	HLA-G polymorphism may play a role in mediating HPV infection risk and induction of cervical lesions	[105]
Ferguson/2012	Canadian	2001–9	CIN II: 159 CIN III: 236 SCC: 144 Normal: 833	CIN II/III/SCC: 36.5 Normal: 30.6	PCR	HLA-G*01:01:01 wild-type allele heterozygotic form and SCC; and progression from CIN to SCC	Homozygous HLA-G*01:01:02, HLA-G*01:06 and SCC HLA-G*3′UTR 14-bp *Ins* and SCC	HLA-G polymorphism is an independent risk factor for the development of SCC	[106]
Gillio-Tos/2012	Brazil	2010	CIN II:150 CIN III: 129 Normal: 510	32,15–47	RT-PCR	NE	NE	Spontaneous demethylation of HLA-G does not occur in CIN II/III	[107]
Silva/2013	Brazil	NR	HSIL: 22 SCC: 33 Normal: 50	24–79 HSIL: 45.5 ± 13.1 SCC: 50.0 ± 12.7 Normal: 50.1 ±14.9	PCR	Polymorphism 3′URT *Del*/*Del* and SCC Haplotypes *Del*/*G* and SCC	Polymorphism *Ins* and *Ins*/*Ins* and HSIL/SCC in smokers Genotype *Ins*/*Del* and HSIL in women with a family history of cancer Haplotype *Ins*/G and HSIL	Polymorphisms in the 3´UTR of HLA-G is associated with an increased risk of developing SCC, especially in smokers	[108]
Metcalfe/2013	Canadian	2002–10	548	15–69	PCR	HLA-G*01:04:01 homozygous genotype and HPV alpha group 3 infection duration	HLA-G*01:01:01 and HPV alpha group 1 HLA-G*01:01:02, G*01:04:01, G*01:06 and HSIL (did not reach statistical significance)	HLA-G polymorphisms play a role in the natural history of HPV infection. HLA-G polymorphisms interact differently with the three alpha HPV groups	[109]
Yang/2014	Taiwan	NR	SCC: 31 Normal: 400	SCC: 54.1 ± 13.8 Normal: 55.7 ± 9.4	PCR	None detected	+3142 C/C genotype and C allele and SCC HLA-G +1537 C/C and +3142 C/C genotypes, C allele and HPV-16 C-*Del*-C haplotype and SCC	HLA-G gene is involved in the susceptibility to SCC	[6]
Bortolotti/2014	Italy	NR	Condyloma: 33 CIN I: 14 SCC: 100 Normal: 100	NR	RT-PCR	None detected	14-bp *Del* allele and high-risk HPV infection *Del*/C haplotype and SCC	HLA-G polymorphisms could represent a risk factor for SCC development in HPV positive subjects.	[84]

Abbreviations: CIN = cervical intraepithelial neoplasia, *Del* = deletion, HPV = human *Papillomavirus*, HSIL = high grade SIL, IHC = immunohistochemistry, *Ins* = insertion, LSIL = low grade SIL, mRNA = messenger ribonucleic acid, NE = not evaluated, NR = not reported, PCR = polymerase chain reaction, RT-PCR = real time-PCR, SCC = invasive squamous cell cervical carcinoma, SIL = intraepithelial cervical lesion.

variations in HLA-G expression impact cervical carcinogenesis risk and progression.

5. HLA-G sequence polymorphism in cervical carcinogenesis

Studies examining the relationship between HLA-G polymorphisms and cervical carcinogenesis have only recently been published, dating back only as far back as 2009 (Table 2).

The first study by Simões et al. in 2009 evaluated HLA-G polymorphisms in Brazilian women with squamous intraepithelial lesion (SIL) (68 with low grade SIL-LSIL, 57 with high grade SIL-HSIL, and 94 healthy women without HPV infection or cytological abnormalities). A significant protective association was observed between the presence of the G*01:03 allele and SIL as well as the G*01:01/G*01:04 genotype and HSIL compared with controls. The presence of the HLA-G0104/14-bp *Ins* and *Del* haplotypes conferred susceptibility to SIL compared with controls. In addition, patients with the HLA-G*01:04/14-bp *Ins* haplotype and HPV-16/-18 co-infections were preferentially associated with HSIL. These results showed an association between HLA-G polymorphisms and HPV infection and SIL, possibly demonstrating a profile compatible with SCC predisposition [104].

In 2011, Ferguson et al. studied the association between HLA-G polymorphisms and HPV infection susceptibility and persistence in 636 female university students in Montreal, Canada. The HLA-G*01:01:02 and HLA-G*01:01:08 alleles were significantly associated with an increased risk of HPV-16 infection acquisition and persistence as well as infection with any of the following HPV alpha species: 1, 8, 10, or 13. The HLA-G*01:01:02 and HLA-G*01:03 alleles were significantly associated with persistent HPV-16 infection and persistent infections from HPV alpha genotypes 2, 3, 4, and 15. These data indicate that HLA-G molecules might play a role in mediating HPV infection risk and the presence of various HLA-G polymorphisms may potentially impact the development of cervical lesions [105].

In 2012, the same research group (Ferguson et al.) published another study focused on the impact of HLA-G polymorphisms on the risk of HSIL and SCC development in a second Canadian population (159 with CIN II, 236 with CIN III, 144 with SCC, and 833 with normal cytology). The wild type HLA-G*01:01:01 allele conferred protection against SCC, whereas variant HLA-G*01:01:02, -*01:06 and -G*-G* 3′UTR 14-bp *Ins* alleles were found to increase SCC risk, after adjusting for age, HPV infection, and ethnicity. These associations were also observed during progression of disease from CIN III to SCC among HPV-positive women. However, HLA-G polymorphisms were not associated per se with CIN III or HPV infection, suggesting that HLA-G plays an important role in the progression of the disease from pre-invasive to invasive SCC. Nevertheless, HLA-G polymorphisms appear to be a strong and independent risk factor for the development of SCC, providing evidence to support the implication of HLA-G molecules in shaping the tumor microenvironment, thus allowing escape from immune responses [106]. This study showed a discrepancy with other studies that observed a positive association between high-risk (HR)-HPV and HLA-G polymorphisms [104,105], which could potentially be explained by differences in the study designs and populations examined. For example, this case–control study investigated factors associated with SCC in older women (mean age 30.6 years), among whom the rate of new infection is generally lower and the presence of HPV is more likely to be a persistent infection or re-infection.

Another case–control study by Gillio-Tos et al. in 2012 evaluated the role of epigenetic modifications (promoter demethylation) of the HLA-G gene on the susceptibility to HPV infection and the development of CIN II/III. This study was performed in samples from Brazilian women (510 with normal cervical cytology, 150 with CIN II, and 129 with CIN III). The methylation analysis of seven cytosine guanine dinucleotides (CpGs) in the HLA-G promoter did not reveal any spontaneous demethylation events in CIN II/III cases (mean proportion of methylation 75.8%) compared to controls (mean 73.7%). The authors concluded that this study did not support the hypothesis that spontaneous demethylation events in the HLA-G promoter are critical for promoting the escape from immunosurveillance in the development of pre-cancerous cervical lesions [107]. Of note, this pilot study did not include SCC samples in their analysis, which may have influenced conclusions drawn from these results.

More recently, Silva et al. (2013) studied the influence of the two HLA-G polymorphisms located in the 3′UTR (14-bp *Ins*/*Del* and +3142*C*/*G*) on the susceptibility to SCC including additional risk factors in samples from Brazilian women (50 samples with normal cytology, 22 HSIL, and 33 SCC). The polymorphism *Del*/*Del* was associated with a decreased risk for developing SCC in smokers, and *Ins* and *Ins*/*Ins* were associated with an increased risk of HSIL and SCC in smokers. The genotype *Ins*/*Del* was associated with an increased risk for HSIL exclusively among women with a family history of cancer. The haplotypes *Ins*/*G* and *Del*/*G* were associated with an increased and decreased risk of HSIL and SCC, respectively. Therefore, the 3′UTR region of HLA-G was found to be associated with an increased risk for developing SCC, especially in smokers [108].

Also in 2013, Metcalfe et al. analyzed the associations among HLA-G polymorphisms, HPV infection, and SIL in 548 Inuit women from Nunavik in Northern Quebec, Canada. In this study, HPV genotypes were classified according to tissue-tropism groupings of alpha-HPV species (α-HPV) as follows: α group 1 included low-risk (LR) cervical genotypes, α group 2 included HR cervical genotypes, and α group 3 included LR vaginal genotypes. HLA-G*01:01:01 was significantly associated with an increased risk of infection duration in α group 1. The homozygous HLA-G*01:04:01 genotype was associated with a decreased risk of HPV infection prevalence in α group 3. No HLA-G alleles were significantly associated with HPV persistence. HLA-G*01:01:02, G*01:04:01, and G*01:06 were associated with HSIL, but the association did not achieve statistical significance. These results suggest that HLA-G polymorphisms may play a role in the natural history of HPV infection, likely at the phase of host immune recognition which differs based on the three α HPV groups [109].

To date, two studies meeting our criteria for inclusion in the review were published in 2014. The first study by Yang et al. enrolled samples from Taiwanese women (317 SCC and 400 healthy controls) and analyzed the HLA-G +1537 A/C, 14-bp *Del*/*Ins*, and HLA-G +3142G/C polymorphisms. The HLA-G +3142 C/C genotype and the C allele were significantly associated with an increased risk for SCC. When the analysis was restricted to the subgroup of women with HPV-16-positive SCC, an association with the HLA-G +1537 C/C and +3142 C/C genotypes and C allele was observed. Moreover, the analysis of haplotype distribution revealed that the +1537 C-14-bp *Del*- + 3142 C haplotype conferred a risk in SCC patients, and the risk further increased in SCC patients infected with HPV-16, suggesting the HLA-G gene plays an important role in the pathogenesis of SCC [6].

The second study in 2014 by Bortolotti et al. analyzed the frequencies of two HLA-G 3′UTR polymorphisms (14-bp *Ins*/*Del*, +3142C > G) involved in HLA-G modulation in samples from Italian women (33 condyloma acuminatum, 14 LSIL, 100 SCC and 100 healthy controls). The 14-bp *Del* allele was shown to be associated with a higher rate of HR-HPV infection, and the *Del*/C haplotype was associated with SCC development. These data indicate that HLA-G polymorphisms may represent a risk factor for cervical disease development in HR-HPV-positive subjects [84].

6. Concluding remarks and future directions

Cervical cancer remains a leading cause of morbidity and mortality for women worldwide. There is a need for robust and sensitive biomarkers to optimize screening methods and treatments, especially in developing countries where a large number of women are already infected with HPV and mortality is heavily impacted by the timing of diagnosis of neoplasias. Based on the data we reviewed, we propose

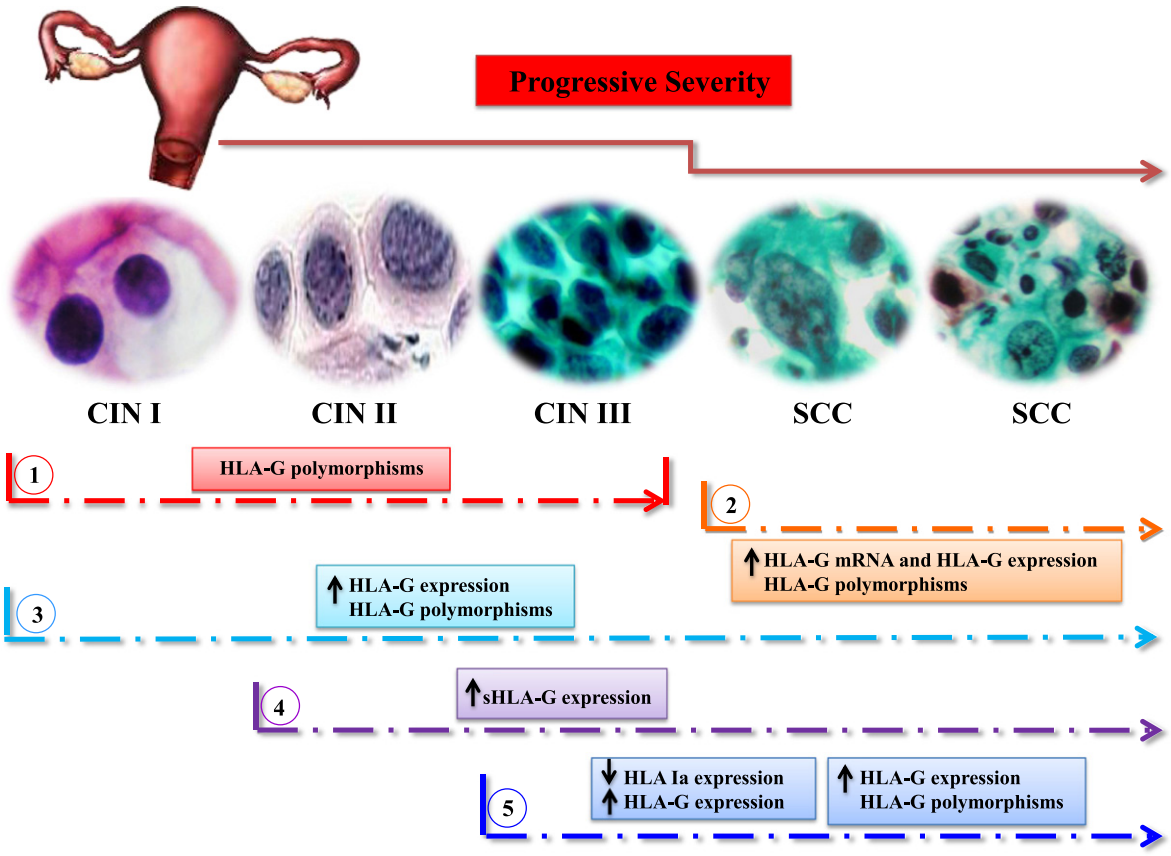

Fig. 6. Schematic representation of possible tolerogenic functions of HLA-G overexpression and polymorphisms in cervical lesions progression and squamous cell cervical carcinoma (SCC) development. 1) HLA-G polymorphisms associated with CIN I progression to CIN III; 2) HLA-G mRNA and HLA-G overexpression [97], and polymorphisms associated with SCC [6]; 3) HLA-G overexpression [65,101] and polymorphisms [84,104] associated with CIN I progression to SCC; 4) sHLA-G expression associated with CIN II progression to SCC [101]; 5) HLA-G overexpression and HLA Ia downregulation associated with CIN III progression to SCC [103]; HLA-G overexpression [102] and polymorphisms [106,1008] associated with CIN III progression to SCC. CIN I to SCC pictures: photomicrographs of cervical smears stained by Papanicolaou under common optical microscopy (40X objective); CIN, cervical intraephelial neoplasia; sHLA-G, HLA-G.

that HLA-G whose engagement generates inhibitory signals in various immune cells, participates in the progression of cervical disease and ultimate tumor cell escape from immunosurveillance (Fig. 6). We theorize that sHLA-G is an important diagnostic/prognostic biomarker for identifying cervical cancer and monitoring disease stage, including assessing the risk of progression of cervical lesions. The clinical value of HLA-G expression stems from its predictive power regarding clinical outcomes in cervical lesions and cancer. Thus, HLA-G tests (e.g., enzyme-linked immunosorbent assay- Elisa or quantitative RT-PCR) are expected to provide physicians with a new molecular approach to better manage patients at risk of developing cervical cancer. Conversely, although recent studies reveal a relationship between HLA-G overexpression and cervical cancer immunoediting that favors the progression of cervical lesions to more advanced stages of invasive cervical cancer, additional studies should be performed to confirm these results.

The majority of studies revealed the involvement of HLA-G polymorphisms in HPV infection and lesion development. Given the low prevalence of HLA-G polymorphisms in the world, HLA-G may be useful as a risk and/or progression biomarker in specific populations. Distinct origin posttranscriptional control mechanisms of HLA-G have recently been suggested. For instance, different HLA-G-specific miRs have been identified that were able to downregulate HLA-G surface expression and miR-mediated inhibition of HLA-G was shown to enhance NK cell recognition [110]. Hence, we postulate that HLA-G-specific miRs might be used as prognostic markers as well as potential therapeutics for targeting HLA-G-expressing cervical cancer. These data support the potential application of HLA-G blockers, such as HLA-G neutralizing antibodies or soluble recombinant LILRB1, LILRB2 or Fas ligand type-II transmembrane protein to the tumor necrosis factor (TNF) family (FasL), inhibiting the binding of HLA-G to LILRB1 or LILRB2 thereby acting as therapeutic agents to minimize inhibitory immune effects.

Another important feature highlighted by Sheu & Shih [17] is that several anticancer drugs induce cancer cells to express increased levels of HLA-G protein, resulting in tumor evasion of the host immune system. For example, 5-aza-2′-deoxycytidine, a demethylating agent used for cancer epigenetic therapy was shown to reactivate HLA-G protein expression in all cell lines tested. Similarly, interferon (IFN) immunotherapy in malignant tumors can drive immune evasion by upregulating the expression of HLA-G at tumor sites [17]. The screening of cervical lesions and cancer cells for HLA-G expression might therefore represent a useful strategy to identify patients who are likely to benefit from epigenetic and IFN therapy.

Thus, in the near future, further studies should be conducted to clarify conflicting points regarding HLA-G in cervical carcinogenesis as well as its application in screening, diagnosis, and risk of progression. These studies will further promote the development of more targeted and effective treatments for this type of cancer.

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