Journal of Reproductive Immunology 166 (2024) 104385

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jri

HLA-G - evolvement from a trophoblast specific marker to a checkpoint molecule in cancer, a narrative review about the specific role in breast- and gynecological cancer

Julia Knabl [a,b], Yao Ye [c], Gernot Desoye [d], Udo Jeschke [e,*]

[a] Department of Gynecology and Obstetrics, University Hospital, LMU Munich, Marchioninistr.15, Munich 81377 , Germany
[b] Department of Obstetrics, Klinik Hallerwiese, St.-Johannis Mühlgasse 19, Nürnberg 90419, Germany
[c] Reproductive Medicine Center, The Affiliated Drum Tower Hospital of Nanjing University School of Medicine, Nanjing, China
[d] Department of Obstetrics and Gynecology, Medical University Graz, Auenbruggerplatz 14, Graz A-8036, Austria
[e] Department of Obstetrics and Gynecology, University Hospital Augsburg, Stenglinstr. 2, Augsburg 86156, Germany

ARTICLE INFO

Keywords:
HLA-G
Cervical cancer
Breast cancer
Ovarian cancer
Endometrial cancer

ABSTRACT

Human leukocyte antigen G (HLA-G) is known as a non-classical molecule of the major histocompatibility complex class Ib and downregulates the mother's immune response against the fetus during pregnancy, thereby generating immune tolerance. Due to the latter effect, HLA-G is also referred to as an immune checkpoint molecule. Originally identified on extravillous trophoblasts, HLA-G is already known to induce immune tolerance at various stages of the immune response, for example through cell differentiation and proliferation, cytolysis and cytokine secretion. Because of these functions, HLA-G is involved in various processes of cancer progression, but a comprehensive review of the role of HLA-G in gynecologic cancers is lacking. Therefore, this review focuses on the existing knowledge of HLA-G in ovarian cancer, endometrial cancer, cervical cancer and breast cancer. HLA-G is predominantly expressed in cancer tissues adjacent to the extravillous trophoblast. Therefore, modulating its expression in the cancer target tissues of cancer patients could be a potential therapeutic approach to treat these diseases.

1. Introduction to Human leucocyte antigen-G (HLA-G)

Placental and cancer genesis are closely related as rapid tissue proliferation in a low oxygen environment are characteristic signs of both entities (Burton et al., 2017). The placenta as well as tumors are protected from the immune system through similar immune escape mechanisms. Several novel immunoregulatory molecules have been discovered over the past few years. One of them is human leukocyte antigen G (HLA-G), which downregulates the host's immune response and thereby generates important immunotolerance.

Human leukocyte antigen G (HLA-G) is a nonclassical major histocompatibility complex Class Ib molecule, located within the major histocompatibility complex (MHC) at chromosomal region 6p21.3. The MHC segment is the most polymorphic region in vertebrate genome (Castelli et al., 2014a). Although the HLA-G product presents the same class I classical molecule structure as the major class I HLA genes (HLA-A, -B, -C), its main function is not antigen presentation. Therefore, the HLA-G gene differs from classical class I genes by its restricted tissue distribution; limited protein variability; presence of several membrane-bound and soluble isoforms; unique molecular structure, presenting a particular peptide-binding groove that impairs peptide presentation to T cells. Only few polymorphic sites are found randomly distributed throughout exons and introns in the coding region, in contrast to the high frequency of polymorphic sites found in classical HLA class I exons (Castelli et al., 2014a).

Over the course of human evolution, the general structure of the molecule was preserved, as evidenced by the conservation of exonic nucleotide sequences encoding residues crucial for molecule dimerization and molecule interaction with leucocyte receptors. However, adjacent nucleotide sequences that function as gene regulatory elements have been identified with several polymorphisms. Nucleotide variability in the promoter regions may alter transcription factor binding affinity, which in turn may affect HLA-G levels. In the HLA-G promoter, there are no IFN-γ or NF-κB, unlike in classical HLA class I genes. In addition, the

* Corresponding author.
E-mail address: udo.jeschke@med.uni-augsburg.de (U. Jeschke).

https://doi.org/10.1016/j.jri.2024.104385
Received 8 April 2024; Received in revised form 7 October 2024; Accepted 15 October 2024
Available online 17 October 2024

proximal promoter region (located within 200 bases from the first translated ATG) does not mediate transactivation by the principal HLA class I transactivation mechanisms; and identified alternative regulatory elements (heat shock, progesterone and hypoxia-responsive elements) as well as unidentified responsive elements for IL-10, glucocorticoids, and other transcription factors are present (Castelli et al., 2014b).

Along with allele type, which controls receptor affinity and expression level (Rebmann et al., 2014), variations in the promoter and 3′ untranslated region (3′UTR) of the gene control the amount of HLA-G expression (Svendsen et al., 2013; Solier et al., 2001). Several single nucleotide polymorphisms (SNPs) in the 3′UTR of the HLA-G gene, in addition to a 14-bp addition or deletion, may have an impact on expression. By attaching to 3′UTR and causing mRNA degradation or translational inhibition, microRNAs (miRs) also contribute to HLA-G mRNA stability. Recent research in the placenta linked HLA-G downregulation to miR-148a and miR-152 (Manaster et al., 2012).

In general, HLA-G induces immunotolerance at different stages of the immune response, for example through cell differentiation and proliferation, cytolysis and cytokine secretion: HLA-G inhibits the cytotoxic response of lymphocytes (Kapasi et al., 2000; Maejima et al., 1997) and NK- and T-cell-mediated cell lysis in the decidua (Rajagopalan and Long, 1999; Rouas-Freiss et al., 1999; Ponte et al., 1999). HLA-G promotes the shift from a proinflammatory Th1 response to a more anti-inflammatory Th2 response (Kapasi et al., 2000; Maejima et al., 1997; Persson et al., 2017).

1.1. HLA-G receptors

HLA-G inhibits immune-modulating activity of different cells. Both soluble and membrane-bound forms bind to inhibitory receptors, such as the immunoglobulin-like transcript (ILT) receptor 2 (CD85j; LILRB1), which is present on lymphoid cells (Shiroishi et al., 2003; Naji et al., 2007), and ILT-4 (CD85d; LILRB2), which is expressed by macrophages, monocytes and dendritic cells (López-Botet and Bellón, 1999; Gao et al., 2000; Fons et al., 2006). In addition, it was also shown that engagement of the ILT2 receptor by HLA-G, expanded the population of myeloid-derived suppressor cells with enhanced suppressive activity (Zhang et al., 2008). Several inhibitory receptors present on natural killer (NK) cells have been shown to bind to HLA-G. HLA-G promotes upregulation of inhibitory receptors on NK cells and CD4+ T cells (LeMaoult et al., 2005). Also, killer cell immunoglobulin-like receptor (KIR) 2DL4/p49 (CD158d) is an HLA-G specific receptor found on NK cells (Yan and Fan, 2005). The killer-cell Ig-like receptor (KIR) 2DL4 (CD158d) acts as an HLA-G receptor and is present on almost all human NK cells (Ueshima et al., 2015). In cell culture models, KIR2DL4 on human mast cells facilitates HLA-G-expressing cancer invasion and subsequent metastasis (Ueshima et al., 2015). Increased numbers of circulating HLA-G(+), IL-10(+), and TGF-β(+) NK cells were found in breast cancer patients, which based on that finding, might impair efficiency of anti-tumor immunity (Ostapchuk et al., 2015).

1.2. Regulation of HLA-G expression

1.2.1. Regulation in ovarian cancer tissue

HLA-G is expressed in a considerable number of ovarian carcinomas at all anatomic sites (Davidson et al., 2005). It is present in effusions of corresponding primary tumors and metastatic lesions of ovarian carcinoma. Its expression levels in tumor cells were significantly lower in effusions obtained during or following chemotherapy. Downregulation after chemotherapy correlated with improved survival (Davidson et al., 2005). Effect of HLA-G downregulation on NK cell cytotoxicity in ovarian tumor cell lines was evaluated. Cells which showed downregulation of HLA-G after transfection with small hairpin RNA (shRNAs) for silencing were better lysed than SKOV3 (ovarian serous cystadenocarcinoma cell line) cells by NK cells. Compared to untransfected cells, shRNA.1-transfected SKOV3 cells were significantly more lysed by NK cells 24 h post-transfection. This might be a first step for cancer therapy by improving immune cell activation (Nazari and Farjadian, 2016).

1.2.2. Regulation in cervical cancer

Higher HLA-G protein levels are found in HPV infected cervix and cervical carcinoma. When soluble and membrane bound HLA-G was analyzed in fresh tissue and plasma of women with HPV-infected and uninfected cervix and cancer cervix specimen using Western blot and ELISA, membrane HLA-G was upregulated in HPV infected cervix and cervical carcinoma in thirty women with cervical carcinoma in comparison to an equal number with normal cervix and 6 with HPV infected cervix. In addition, soluble HLA-G concentration was significantly elevated in cervical cancer patients in comparison to patients with a normal cervix (Aggarwal et al., 2020). This suggests that HLA-G upregulation favors the persistence of HPV in a microenvironment of a submissive host response, and this further seems to support the development of cervical cancer.

1.2.3. Regulation in endometrial cancer

A comprehensive study investigated HLA-G expression and regulation in a large cohort of patients with endometrial cancer (Bijen et al., 2010): Tissues from over 500 patients were stained with monoclonal antibody 4H84 (anti-HLA-G recognizing all isotypes) and markers for classical MHC I molecules (mAB HC-10, which recognizes b2-m-free HLA-B and HLA-C heavy chains and b2-m-free HLA-A10, -A28, -A29, -A30, -A31, -A32 and -A33 heavy chains. The results were linked to known clinic-pathological characteristics such as FIGO stage, tumor type, tumor grading, therapy as well as survival. HLA-G levels were higher in 40 % of cases, whereas lower classical MHC I antigens were found in neoplastic cells in 48 % of cases. HLA-G expression was related to neither clinic-pathological parameters nor survival in this study. Downregulation of MHC I molecules prevents tumor recognition by CTLs and has been found in several malignancies. Absence of HLA-G expression in endometrial cancer was independently associated with MHC class I downregulation, which in turn was a predictor for disease-specific survival, as prognostic unfavorable tumor characteristics were correlated with downregulation of MHC class I (Bijen et al., 2010). Further research is warranted to unravel the regulatory process, as there seems to be a relation between classical and nonclassical MHC class I molecules (HLA-G).

1.2.4. Regulation in breast cancer tissue

Little is known about the molecular regulation of HLA-G expression in breast cancer. Preliminary work showed that progesterone regulates HLA-G expression through a novel progesterone response element in the HLA-G promoter region (Yie et al., 2006). The potential of progesterone to upregulate HLA-G was also demonstrated in breast cancer MCF-7 cells, in which HLA-G expression was enhanced by estradiol/progesterone, but reduced by their antagonists (He et al., 2010). Additional cytotoxicity studies showed that allocytotoxic lymphocyte (allo-CTL) response in MCF-7 cells was inhibited by prior treatment with estradiol/progesterone, but was amplified by their antagonists (He et al., 2010). This may suggest that effects of HLA-G could be restored or further strengthened by the addition of anti-HLA-G antibodies (He et al., 2010).

Also, estradiol (E2) can affect HLA-G expression. E2 through the G-protein coupled estrogen receptor (GPER) induces miR-148a levels in MCF-7 and MDA-MB-231 cells regulating HLA-G expression (Tao et al., 2014). In addition, these findings offer important new insights into the ability of estrogenic GPER signaling to trigger HLA-G expression through inhibiting miR-148a. These functional relationships support immune evasion in breast cancer (Tao et al., 2014).

Gene promoter methylation regulated by TETs and DNMTs may also contribute to HLA-G overexpression of HLA-G in MCF-7 cells (Zhang et al., 2019). Lower DNMT1 and DNMT3a and higher TET2 expression

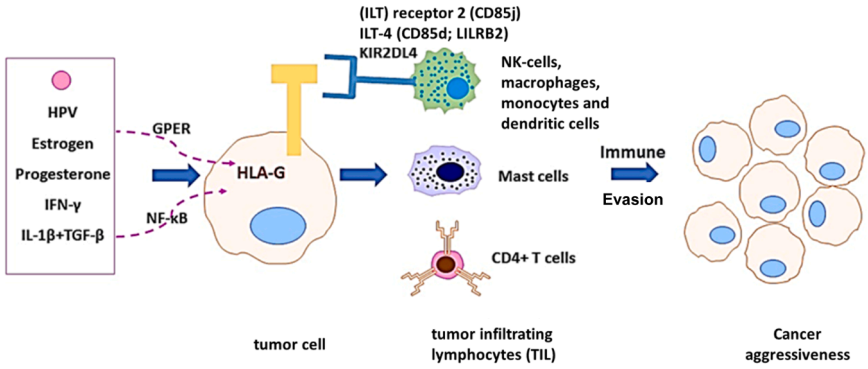

Fig. 1. Expression of HLA-G in tumour tissues has been associated with cancer progression. HPV, estrogen, progesterone, IFN-γ, IL-1βand TGF-β induce HLA-G expression (blue arrow) through different signaling pathways, such as the G-protein coupled estrogen receptor (GPER) and NF-kappa B signaling pathways (both visualized by dotted purple lines). The expression of HLA-G and its receptors (KIR2DL4) in NK cells, (ILT) receptor 2 (CD85j), ILT-4 (CD85d; LILRB2) on macrophages, monocytes, dendritic cells, mast cells and CD4+ T cells facilitate immune evasion, which is associated with increased tumor metastasis and subsequent metastasis.

levels may account for abnormal DNA methylation of HLA-G in MCF-7 (Zhang et al., 2019). Treatment with a TET inhibitor prevented aberrant HLA-G expression and DNA methylation in MCF-7 (Zhang et al., 2019).

Further studies characterized HLA expression in breast cancer and malignant melanoma cell lines and investigated the induction of HLA-G expression by two distinct mechanisms: stimulation with interferon (IFN)-γ or inhibition of methylation by treatment with 5-aza-2′-deoxycytidine (5-aza-dC) (Jorgensen et al., 2020). These studies demonstrated that HLA-G mRNA was elevated upon treatment with 5-aza-dC and a combination of IFN-γ and 5-aza-dC (Jorgensen et al., 2020).

Protein disulfide isomerase A1 (PDIA1) regulates breast cancer cell immunorecognition in a manner dependent on redox state (Alhammad et al., 2020). In particular, addition of PDIA1 to MCF-7 and MDA-MB-231 cells resulted in higher surface levels of HLA-G under oxidative stress conditions (Alhammad et al., 2020).

1.2.5. Physiology and pathophysiology of HLA-G in pregnancy

During pregnancy, the semi-allogenic fetus needs to induce maternal immunotolerance to prevent its rejection. An important role in the special immune suppression system in pregnancy is attributed to the HLA-G (Hunt et al., 2005). It is expressed in placental tissue invading the maternal uterine decidua during implantation, similarly to the invasive growth process seen for tumors.

Its highest expression levels are recognized in extravillous cytotrophoblast cells (EVT) with little, if any, expression in the syncytiotrophoblast (SCT) (O'Callaghan and Bell, 1998; Le Bouteiller and Lenfant, 1996; Le Bouteiller et al., 1996). EVTs are positioned near to maternal immune cells at the maternal-fetal interface, where they can actively contribute to maternal immunotolerance. EVT do not express the classical MHC class I molecules HLA-A and -B. Therefore, the invasive cytotrophoblast is not recognized as non-self by the maternal immune system (Bainbridge et al., 2000, 1999) resulting in the inability to induce immune responses.

As a result of alternative splicing, several HLA-G isoforms exist (Hunt et al., 2005). These mRNA variants encode one full-length isoform (HLA-G1), three short membrane-bound isoforms (HLA-G2, -G3, -G4) and two soluble isoforms (HLA-G5, -G6). HLA-G gene almost completely lacks polymorphisms, thus, there is little inter-individual variation. HLA-G has been implicated in the complex network governing EVT invasion through interaction with uterine NK cells. Several inhibitory receptors present on NK cells and CD4+ cells have been shown to bind HLA-G and this promotes upregulation of inhibitory receptors on NK cells and CD4+ T cells (LeMaoult et al., 2005). Also, epigenetics plays a role in HLA-G expression in pregnancy. Decreased expression of the histone demethylase JHDMID in the placenta contributes to preeclampsia and lower expression of HLA-G (Luo et al., 2018).

HLA-G plays a pivotal role in suppression of immune responses and contributes to long-term immune tolerance. Overall, HLA-G stimulates the shift from pro-inflammatory state, which is important for implantation (Roussev and Coulam, 2007; Le Bouteiller, 2004), to an anti-inflammatory state in later stages of pregnancy. In addition, HLA-G stimulates the secretion of pro-angiogenic factors via NK-cells that promote placental development (Rajagopalan and Long, 1999) and might also be involved in the regulation of angiogenesis and cell migration during formation and maturation of the placenta (Le Bouteiller et al., 2007). Specifically, soluble (s)HLA-G stimulates the release of TNF-α and IFNγ, whereas it inhibits IL-3. Membrane bound HLA-G reduces IL-4, whereas sHLA-G stimulates IL-10 (Kanai et al., 2001).

The restrictive expression pattern of HLA-G in the placenta with a very strong expression in invasive trophoblast cells of the placenta (Kovats et al., 1990; Hackmon et al., 2017) makes it a widely accepted marker for EVT cells in immunofluorescence staining and can also serve as normalization parameter in analyses of total first trimester trophoblast cell preparations.

HLA-G expression is not invariant in the human placenta, but can be altered in disorders of reproduction: Lower HLA-G expression levels were found in early pregnancy failure, i.e., miscarriage and recurrent abortion (Moreau et al., 2009; Quach et al., 2014; Ferreira et al., 2016) and also in pre-eclamptic placentas in comparison to control (Goldman-Wohl et al., 2000; Yie et al., 2004). In gestational diabetes, extravillous trophoblasts contain less HLA-G than in normal pregnancies (Knabl et al., 2023). Physiological and pathological conditions associated with HLA-G extend beyond its expression in placenta or trophoblasts, respectively. Thymus, cornea, and erythroid and endothelial precursors, all exhibit HLA-G expression under physiological conditions (Lefebvre et al., 2000; Menier et al., 2004; Le Discorde et al., 2003). That being said, HLA-G encodes a crucial molecule for the immune system, as evidenced by the association between HLA-G variation sites and/or expression levels and pathological conditions like viral infections (Haddad et al., 2011; Simões et al., 2009), autoimmune diseases (Brenol et al., 2012), transplantation outcome (Mociornita et al., 2013), and inflammatory diseases (Carosella et al., 2001).

1.2.6. HLA-G in cancer development

It is believed that immune-mediated tumor identification, also known as immune editing of cancer, accounts for the ongoing development of tumor phenotypes. The process of immune editing is divided into three stages: elimination (immune surveillance), balancing (duration/dormancy), and escape (progression) (Kim et al., 2007). During the elimination phase, neo-antigens derived from the tumor can induce an immune response that leads to cell death. Those tumor cells that are not

destroyed will continue to grow, leading to a period of equilibrium between tumor and immune cells (balance phase). Finally, the escape phase occurs when the tumor can evolve and changes its environment, thus ultimately evading the immune system and its protective function.

HLA-G has numerous immunosuppressive effects due to its binding and thereby activating various inhibitory receptors on different immune cells (Loustau et al., 2020). The presence of immune checkpoint molecules that suppress macrophages, dendritic cells (DCs) and regulatory T cells (Tregs) induces a tolerogenic environment. HLA-G is one of the checkpoints, which works as an effective way to escape an immune response (Loustau et al., 2020).

These three phases incorporate the immune system's capacity to both prevent cancer in the host and foster the growth of cancer (Dunn et al., 2004). HLA-G takes part in all stages of tumor immune escape (Li et al., 2021).

In the elimination phase, HLA-G acts through inhibiting T and B cell activation and proliferation, by blocking cytotoxic function of T- and NK-cells and by blocking the function of neutrophils and dendritic cells (Morandi et al., 2010; Le Gal et al., 1999; Menier et al., 2002; Favier et al., 2010). During the equilibrium phase it downregulates MHC class II expression on DCs and induces suppressive myeloid cells (Ristich et al., 2005). During the escape phase, hypoxia induces upregulation of VEGF, HIF-1, and HLA-G expression, and immunosuppressive cytokines, such as IL-10 and TGF-β, are secreted (Muz et al., 2015; Moreau et al., 1999).

HLA-G can be preferentially detected in tumor tissue, *in situ* but is absent in surrounding normal tissue in a variety of malignancies. Its expression is strongly associated with tumor progression or metastatic status and with a poor prognosis. However, underlying mechanisms remain to be explored (Lin et al., 2013; Gonzalez et al., 2012).

HLA-G is expressed in various cancers (Rajagopalan and Long, 1999; Fainardi et al., 2011). HLA-G overexpression in malignant tissues of was first identified in choriocarcinomas and cancerous trophoblastic cells (Ellis, 1990), and later in malignant melanoma (Paul et al., 1999). After that, HLA-G expression has also been observed in various cancers at almost all anatomic sites (e.g. gastro-intestinal tract, lungs, central nervous system etc.). HLA-G expression levels are closely related to the immunosuppressive microenvironments, advanced staging and adverse outcome (Lin and Yan, 2018; Ben Yahia et al., 2018; Babay et al., 2018).

The mechanisms of inhibited immune response of the host against tumor involve disorders of number and function of immune cells, and altered expression of cellular membrane molecules, which prevents the tumor to be recognized from and eliminated by host immune cells.

Therefore, the abnormal expression of HLA-G in tumors is one of the main contributors to immune escape and tumor progression. In the following sections we describe what is known about the role of HLA-G in gynecologic oncology.

1.2.7. Ovarian cancer

Ovarian cancer is one of the most aggressive gynecologic cancers and a leading cause of death from gynecologic malignancies in the developed world. Over 90 % of ovarian cancers are classified as epithelial ovarian cancer, e.g., as serous, mucinous, endometroid, clear cell, and transitional cell types.

Immune deficiencies in the ovarian tumor environment support progression of the tumor (Torres et al., 2009). One of the mechanisms that ovarian cancer cells evade immune surveillance is by upregulating human leukocyte antigen-G (HLA-G) expression (Jung et al., 2009; Menier et al., 2009).

Early studies (Jung et al., 2009) hinted at HLA-G expression to play a pivotal role in development of epithelial ovarian cancer and disease progression. These studies found enhanced HLA-G mRNA expression in ovarian cancer paralleled by higher protein levels. Interestingly, both HLA-G mRNA and protein levels were significantly greater in advanced ovarian cancer tissues than in early-stage ovarian cancer tissues. Thus, patients with elevated HLA-G expression had a worse prognosis, as HLA-G immunoreactivity and patient survival correlated significantly (Jung et al., 2009). Menier et al. looked for HLA-G expression in ovarian carcinoma lesions from low to high grade and stage and showed that HLA-G is selectively expressed in advanced-stage disease of high-grade histology (Menier et al., 2009).

HLA-G expression is frequently detected in the most aggressive type of ovarian cancer, i.e., high-grade serous carcinoma (Sheu and Shih, 2007).

HLA-G plays a key role in evasion of ovarian cancer cells from immunosurveillance: cytotoxicity studies showed that HLA-G expression inhibits cell lyses by NK-92 cells. Importantly, cell lysis could be restored by the anti-HLA-G monoclonal antibody 87 G, specific for HLA-G (Kim et al., 2007).

HLA-G has a potential association with progressive disease (Babay et al., 2018). Its presence in tumor tissue was a promising candidate parameter to predict disease recurrence and it correlated with advanced stages of ovarian carcinoma. HLA-G and HLA-E are frequently co-expressed, and both are present in most ovarian carcinoma tissues (Babay et al., 2018).

In contrast, HLA-G expression was also found a valuable prognostic factor for improved survival in high grade epithelial ovarian cancer and a predictor for sensitivity for platinum containing chemotherapies (Rutten et al., 2014).

The monoclonal antibody (mAb) 4H84 was widely used in earlier studies. Different HLA-G isoforms might have different biological functions in malignancies. This is why specific HLA-G isoforms such as HLA-G5/-G6 were studied in 118 primary ovarian cancer lesions (mAb 5A6G7 by immunohistochemistry). However, HLA-G5/-G6 immunoreactivity in lesions was unrelated to histological type, patient age, FIGO stage and patient survival. Thus, HLA-G5/-G6 protein levels have no clinical significance in ovarian cancer lesions (Zhang et al., 2016).

In a murine model of ovarian cancer, HLA-G levels were associated with increased tumor metastasis and with poor survival. Cell migration and invasion capability increased in ovarian carcinoma cell lines (HO-8910 and Ovcar-3) that had been transfected with HLA-G gene in a mouse model (HO-8910-G and Ovar-3-G). These cell lines were of higher invasion potential and showed widespread metastasis in mice xenografted with HO-8910-G cells and mouse survival was profoundly decreased in mice xenografted with HO-8919-G and OVAR-2-G cells (Lin et al., 2012).

HLA-G effects on tumor invasiveness and metastasis may rely on inhibition of NK cytotoxicity and on induction of MMP-15 expression. HLA-G expression decreased NK cytotoxicity against the ovarian carcinoma cell line (HO-8910-G) and upregulated MMP-15 in these cells. HLA-G and MMP-15 expression was strongly positively correlated in ovarian cancer tissue. In addition, knockdown of the HLA-G induced MMP-15 expression significantly resulted in decreased migration potential of the ovarian carcinoma cell line HO-8910-G (Lin et al., 2013).

The potential role of DNA methylation on HLA-G expression in ovarian cancer, was studied (Menier et al., 2009 (Menendez et al., 2008)). A region having an intact hypoxia response element (HRE) remained completely methylated in the ovarian cancer cell line BG-1 after treatment with 5-aza-deoxycytidine (5-aza-dC) and was completely methylated in all the ovarian tumor (malignant and benign) samples examined but was only variably methylated in normal ovarian surface epithelial cells. HLA-G expression was significantly increased in the 5-aza-dC treated cell line, but no significant difference in HLA-G expression was detected between the tumor and normal samples. Since HRE is the binding site of a known repressor of HLA-G expression (HIF-1), methylation of the region surrounding the HRE keeps the potential for expression of HLA-G in ovarian tumors. The influence of hypoxia on HLA-G gene induction had already been demonstrated in choriocarcinoma and melanoma cell lines (Mouillot et al., 2007).

Menendez et al. found no consistent or significant differences in HLA-G expression between the OSE and tumor samples as HLA-G expression levels are highly variable among all samples. Therefore, the

authors concluded that changes in methylation could be necessary, but not sufficient, for HLA-G expression in ovarian cancer (Menendez et al., 2008).

As single nucleotide polymorphisms (SNPs) regulate HLA-G expression (c.f. introduction above), certain SNPs have the potential to contribute to different outcome in epithelial ovarian cancer. HLA-G 3′UTR haplotypes UTR-1 or UTR-2 were significantly associated with metastasis, whereas the UTR-5 and UTR-7 haplotypes were significantly associated with a better prognosis (Schwich et al., 2019).

Soluble HLA-G concentration is significantly higher in malignant ascites (Singer et al., 2003). HLA-G synthesis is associated with the presence of inflammatory cytokines and is strongly correlated with Tregs and diminution of NK- and memory T-cells. Interleukin-1ß along with TGF-ß are capable of inducing HLA-G expression through the NF-kappaB pathway (Ullah et al., 2019).

1.2.8. Cervical cancer

Human Papillomavirus (HPV) is a considerable risk-factor for cancer of the cervix. However, persistent HPV infection results in cervical cancer in only a minority of patients. It seems that HPV infection predisposes the development of cervical cancer through a multi-stage tumorigenic process: localized immune dysfunction characterized by reduction of CD4 T-helper lymphocytes in lesions of HPV-associated carcinomas, and regression of HPV-induced lesions accompanied by tissue infiltration with macrophages, cytotoxic T cells, and natural killer cells (Viac et al., 1993).

As HLA-G has been found to execute major tolerogenic functions, the hypothesis was generated that HPV suppresses the host immune response, which includes overexpression of HLA-G and leads to progression to cervical cancer (Dong et al., 2010). On the other hand, low expression of soluble HLA-G5 was found in invasive cervical cancer with and without metastasis, associated with HPV-positivity (Guimaraes et al., 2010). A Chinese study found that both HLA-G expression in situ and serum sHLA-G levels were dramatically increased in patients with cervical premalignant and malignant lesions (Zheng et al., 2011). Another Chinese study stated that HLA-G expression increased progressively from pre-malignant to malignant cervical lesions. This group speculated that their findings suggests that HLA-G expression induced in cervical cancer cells might be an additional mechanism for tumor cells evading from host immunosurveillance (Li et al., 2012).

Already early investigations showed that HLA-G polymorphisms may be associated with HPV infection and squamous intraepithelial lesions and therefore representing a profile of predisposition to cervical cancer (Simões et al., 2009). A study from Brazil found that the HLA-G 3′UTR polymorphisms (specially the 14 bp *In allele and *In/G haplotype) may be associated with a greater risk of cervical cancer development among HPV-infected and smoker patients (Silva et al., 2013). Data from a Canadian study group support the theory that HLA-G polymorphisms play a role in the natural history of HPV infections. HLA-G polymorphisms interact differently with different alpha papillomavirus groups and they may play a greater role in viral acquisition than viral clearance (Metcalfe et al., 2013). A group from Taiwan showed that the C/C genotype and C allele of the HLA-G +3142 SNP are significantly associated with CSCC risk. In addition, patients possessing the +3142 and +1537 C/C genotype and C allele and harboring HPV-16 infection are more susceptible to CSCC. This genotype and allele might render a female more vulnerable to persistent HPV infections and the development of CSCC (Yang et al., 2014). Interestingly, an Italian study showed a primary association between HPV infection development and HLA-G 3′UTR polymorphisms, where the simultaneous presence of high-risk HPV infection and HLA-G high production genetic background promotes the neoplastic progression (Bortolotti et al., 2014). Another more recent study investigated the association of HLA-G 3′ UTR polymorphism and expression with the progression of cervical lesions in human papillomavirus 18 infections (Xu et al., 2018). They found that the HLA-G polymorphisms (alleles *14 bp In* and + 3142 G) are also associated with the progression of HPV18-related cervical lesions (Xu et al., 2018). The same group published a new prognostic risk model for cervical cancer based on immune checkpoint HLA-G-driven differentially expressed genes (Xu et al., 2022). HLA-G-driven DEG signature consists of eight most important prognostic genes CD46, LGALS9, PGM1, SPRY4, CACNB3, PLIN2, MSMO1, and DAGLB that were identified as a key predictor of cervical cancer (Xu et al., 2022). Another recent study of this group found dynamic changes of soluble HLA-G and cytokine plasma levels in cervical cancer patients and discussed its potential role in cancer progression and immunotherapy (Xu et al., 2023). In addition, HLA-G 3′UTR polymorphism diplo-types and soluble HLA-G plasma levels that impact cervical cancer susceptibility and prognosis were also investigated by the same group of researchers (Gan et al., 2022).

In an HPV+-subgroup, the effect of human leukocyte antigen G alleles on human papillomavirus infection and persistence were investigated in a cohort of HIV-positive pregnant women from Brazil (Alves et al., 2015). In this specific subgroup the authors found a protective effect against the occurrence of cytological abnormalities in patients carrying the G*01:01:02 allele (Alves et al., 2015). A further study on a similar group showed that in HIV+ women the +3142 C allele and the +3142 CC genotype, which are related to increased sHLA-G production, were associated to the presence of aneuploidy in cervical cells in an allele dose-dependent effect (Medeiros et al., 2018). Therefore, the authors concluded that the +3142C allele is associated to HPV infection and to a higher risk to develop cervical cancer (Medeiros et al., 2018). Another investigation of this group referred to the contribution of HLA-G and FOXP3 genes and proteins in the severity of cervical intraepithelial neoplasia during HPV infection (da Silva et al., 2023). They found that sHLA-G+ cells were positively correlated to Foxp3+ cells in presence of HPV infection and in cervical grade II/III injuries. Therefore, HPV may use HLA-G and Foxp3 as a way of host immune escape contributing to the persistence of infection and inflammation, leading to the cervical lesion and the worsening of lesions (da Silva et al., 2023).

HLA-G and IL-17 immunoreactivity was elevated in specimens that showed cervical intra-epithelial neoplasia grade I, thus suggesting that these molecules contribute towards progression in cervical neoplasia (Miranda et al., 2015). A Chinese study showed that long non-coding RNA HOTAIR modulates HLA-G expression by absorbing miR-148a in human cervical cancer (Sun et al., 2016). Another group investigated HLA-G expression by real time PCR and host immune response by counting the number of tumor-infiltrating lymphocyte (TIL) and NK CD57+ cells. HLA-G expression increased progressively from precancerous and cancerous cervical lesions. There was an inverse relationship between HLA-G expression and estimated number of TILs and NK CD57+ cells (Fahim et al., 2018).

While other groups found a stronger protein expression of HLA-G in CIN and cervical carcinoma, the group of Zhou et al. found strong and uniform immunohistochemical staining of HLA-G in ectocervical squamous and endocervical columnar regular epithelium. Compared with the results in control samples, CINs and squamous cell carcinomas showed significantly reduced HLA-G levels (Zhou et al., 2006). A Dutch group investigated classical and non-classical HLA class I aberrations in primary cervical squamous- (SCC) and adenocarcinomas and paired lymph node metastases (Ferns et al., 2016). They found that tumor immune escape variants leading to metastasis. Moreover, SCC tumors showing downregulation of HLA-A or total classical HLA in combination with HLA-G expression had poor prognosis (Ferns et al., 2016). A Japanese group described a new approach to finding targets for cervical cancer stem cell treatment by investigating the regeneration of cervical reserve cell-like cells from human induced pluripotent stem cells (iRCs) (Sato et al., 2017). They found that iRCs secreted higher levels of several inflammatory cytokines such as macrophage migration inhibitory factor (MIF), soluble intercellular adhesion molecule 1 (sICAM-1) and C-X-C motif ligand 10 (CXCL-10) compared with normal cervical epithelial cells. These cells also expressed human leukocyte antigen-G (HLA-G), which is an important cell-surface antigen for immune tolerance and

carcinogenesis (Sato et al., 2017).

1.2.9. Endometrial cancer

Endometrial adenocarcinoma is the most common gynecological malignancy in the developed world. As most patients are diagnosed at an early disease stage, the overall prognosis is good. However, prognosis gets worse at higher stages. Non-endometrioid histological type or Grade 3 endometrioid carcinoma have been identified as prognostically worst subgroups (Bijen et al., 2010).

HLA-G protein is found in a considerable number of endometrial adenocarcinomas, in which it is mostly located in the glandular, but not stromal, epithelium. Analysis of 44 primary endometrial adenocarcinomas using immunohistochemical staining with the 4H84 anti-HLA-G monoclonal antibody found a significant correlation between HLA-G protein staining intensity and immunopositively cells and increasing stage of endometrial cancer. These results were also found at the mRNA level (Barrier et al., 2006).

Comparable results had been found by other groups: HLA-G levels were elevated in endometrial carcinoma compared to adjacent normal endometrial tissues, especially in grade 3 endometrial carcinoma and in the non-endometrioid type 2 endometrial carcinoma (Ben Yahia et al., 2020). Higher HLA-G levels in tissue samples from women with advanced endometrial cancer than in tissue from early-stage disease (Walentowicz-Sadlecka et al., 2019) confirmed earlier findings. In addition, when metastases were found in lymph nodes of the primary tumor, HLA-G levels were significantly elevated (Walentowicz-Sadlecka et al., 2019).

These results suggest that HLA-G levels could be used to stage endometrial cancers and to indicate their prognosis. The presence of HLA-G within the cancer and its surroundings seems associated with disease progression.

Also, sHLA-G plasma levels were significantly increased in patients with endometrial carcinoma compared to healthy controls. The majority of sHLA-G in plasma from endometrial carcinoma patients was present in monomeric form. sHLA-G has been shown to be increased in initial stages as well as in Grade 3 endometrial carcinoma (Ben Yahia et al., 2018).

2. Breast cancer

2.1. Introduction

HLA-G is expressed by the hormone receptor positive breast cancer cell line MCF-7 (Pangault et al., 1999). In addition, HLA-G is also present on tumor associated macrophages (Pangault et al., 1999). These initial findings prompted a large number of further studies on expression, prognostic value, immunological role and on regulation of HLA-G in breast cancer. This chapter aims to describe the latter roles for HLA-G in breast cancer.

2.2. Expression of HLA-G in breast cancer tissue

At about the same time when HLA-G was found in MCF-7 cells, HLA-G was also demonstrated in breast cancer tissue (Lefebvre et al., 2002). Immunohistochemical analysis of cryopreserved and paraffin-embedded breast tissue biopsies using two HLA-G specific antibodies found that 14 of 36 breast cancer lesions exclusively expressed HLA-G as opposed to non-cancerous breast tissue surrounding the tumor. HLA-G protein was significantly more abundant in lesions heavily infiltrated by host immune cells compared to regions with low immune infiltration, suggesting that HLA-G activation correlates with inflammation. Further histological and double immunofluorescence analyzes demonstrated HLA-G mainly on tumor epithelial cells and on subsets of infiltrating CD68+ and CD8+ cells (Lefebvre et al., 2002). A subsequent study confirmed these findings. On the basis of immunohistochemistry, 22 (25 %) invasive ductal carcinomas of the breast were HLA-G immunoreactive with between 2 % and 100 % of the tumor cells being immune-positive for HLA-G (Singer et al., 2003). cDNA microarrays identified genes differentially expressed between lobular and ductal tumors of the breast (Korkola et al., 2003). Prediction analysis for microarrays (PAM) was able to predict tumor type with an accuracy of 93.7 %. HLA-G was among the 3 genes that were identified by significance analysis for microarrays and PAM, the others were osteopontin and CHC1 (Korkola et al., 2003). These results were confirmed by a study, which found 8 genes differentially expressed between lobular and ductal cancers: In addition to confirming HLA-G, osteopontin and CHC1 (Korkola et al., 2003), also E-CD, survivin, cathepsin B, TPI1, SPRY1, SCYA14, TFAP2B, and thrombospondin 4 were found, (Turashvili et al., 2005).

In malignant mesothelioma and breast carcinoma, HLA-G is only focally expressed, while HLA-ABC expression is not down regulated (Kleinberg et al., 2006). Up-regulated expression of HLA-G in malignant mesothelioma effusions and its association with shorter disease-free survival time in advanced stage of breast carcinoma suggest a possible role in immune response evasion in these tumors (Kleinberg et al., 2006). Another study showed that approximately 66 % of neoplasmic lesions of the breast had HLA-G protein expression. Expression levels were significantly correlated with tumor size, nodal status, and clinical disease stage (He et al., 2010). Patients with HLA-G expression had a lower survival rate than those with no HLA-G, and plasma sHLA-G levels were significantly higher in breast cancer patients than in healthy controls (He et al., 2010). A study on HLA-G expression and prognoses demonstrated that patients with loss of classical HLA class I tumor protein expression, expression of HLA-E, HLA-G, or HLA-EG resulted in a shorter relapse-free period (de Kruijf et al., 2010). In a recent study, the prognostic and predictive value of HLA-G and HLA-F protein isoform expression patterns in patients with breast cancer were evaluated (Wuerfel et al., 2020). Presence of distinct HLA-G isoform proteins, in particular HLA-G6, could serve as a possible new marker for pathological complete response in HER2+ breast cancer (Wuerfel et al., 2020).

2.3. The role of soluble HLA-G as a breast cancer tumor marker

A preliminary study determined whether soluble HLA-G (sHLA-G) could serve as a marker for malignant ascites in breast and ovarian cancer, the most common malignancies causing ascites in women. Soluble- HLA-G concentration measured in 42 malignant and 18 benign ascites supernatants was significantly higher in malignant ascites than in benign controls (Singer et al., 2003). Increased sHLA-G plasma levels were also found in patients suffering from malignant melanoma, glioma, breast and ovarian cancer (Rebmann et al., 2003). Specific ELISpot assays demonstrated that sHLA-G molecules transcribed from intron-4 sequences are preferentially secreted by peripheral blood monocytes in comparison to other sequences (Rebmann et al., 2003). Later studies reported that sHLA-G may be used as a tumor marker in breast cancer patients (Sayed et al., 2010). HLA-G may also play critical role in the progression of breast cancer, and plasma sHLA-G levels might be a useful pre-operative biomarker for breast cancer diagnosis (Chen et al., 2010). Patients with mixed type of coexisting ductal and lobular breast lesions had significantly increased sHLA-G concentration as compared to patients with pure ductal carcinoma or pure lobular neoplasia (Provatopoulou et al., 2012). Soluble HLA-G levels in plasma samples from women with breast cancer were correlated with pregnancy and breastfeeding history (Zidi et al., 2016a). These results indicate potential implication of previous pregnancy and breastfeeding experience in sHLA-G expression during breast cancer (Zidi et al., 2016a). Soluble HLA-G was also tested as prognostic marker for the prediction of clinical outcome of neoadjuvant chemotherapy (NACT) in breast cancer patients (Konig et al., 2016). Within that study different sHLA-G variants were variable qualitative prognostic value for assessing potential clinical outcome of NACT treated BC patients (Konig et al., 2016). Moreover, elevated sHLA-G may be related with advanced stages of breast cancer

(Zidi et al., 2016b).

2.4. HLA-G polymorphism and its prognostic relevance

The possible influence of HLA-G polymorphisms on susceptibility to breast cancer development was focus of some studies (Table 1). Brazilian subjects In Brazilian subjects the variation in the HLA-G coding region was not a risk factor for breast cancer (Rolfsen et al., 2014). This contrasted another study, which evaluated the association of 14-bp ins/del polymorphism in HLA-G gene and breast cancer in a south-east Iranian population (Eskandari-Nasab et al., 2013). The results suggested that this polymorphism could be a genetic risk factor for the susceptibility to breast carcinoma (Eskandari-Nasab et al., 2013). The frequency of the 14-bp insertion/deletion HLA-G polymorphism, as well as the expression of this molecule was studied in patients with invasive breast ductal carcinoma (IDC) (Ramos et al., 2014). The hypothesis tested was that the polymorphism observed in patients with IDC induces higher HLA-G protein molecules, which may possibly contribute to shorter survival time and a worse clinical prognosis in these patients (Ramos et al., 2014). The presence of a 14-bp sequence in the 3′-untranslated region (UTR) of HLA-G is associated with a lower risk of breast cancer susceptibility based on HLA-G expression in breast cancer tissues (Jeong et al., 2014). These results differ from another study in which the HLA-G 14-bp ins/del polymorphism was associated with breast cancer susceptibility (Li et al., 2015). This polymorphism is a potential genetic risk factor also for breast cancer progression (Haghi et al., 2015). When 104 patients with breast cancer and 83 controls (CTRL) were genotyped for HLA-G 14-bp insertion/deletion (Ins/Del) and HLA-G +3142 C>G polymorphisms in a Tunisian population and compared the results with HLA-G gene dosage (Zidi et al., 2016b), HLA-G +3142 C>G polymorphism associated with breast cancer susceptibility. A further study analyzed three HLA-G 3′UTR potential polymorphisms: +3187 A > G (rs9380142), +3142 G > C (rs1063320), +2960 14-base pair (bp) Insertion/Deletion (Ins/Del) (rs66554220), and the HLA-E*01:01/01:03 A > G (rs1264457) polymorphism in a Tunisian breast cancer population (Ouni et al., 2019). The +3142 C allele and +3142 C/C genotype was significantly associated with increased breast cancer risk (Ouni et al., 2019). A recent meta-analysis of HLA-G 14-bp Ins/Del polymorphism and how it relates to cancer risk, found an important role particularly in breast cancer (Jiang et al., 2019) in comparison to other cancer forms.

When the associations of HLA-G 3′UTR (14-bp Ins/Del and +3142 C/G) and TNF-α promoter (-238 G/A and −308 G/A) polymorphisms on breast cancer risk were studied among South Indian women (Kadiam et al., 2020), significantly higher frequencies of HLA-G 14-bp Ins allele and Ins/+3142 G haplotype were observed in breast cancer patients than in healthy controls (Kadiam et al., 2020). Analyses of links between any of eight (UTR) single nucleotide polymorphisms (SNPs) and their HLA-G gene haplotype with breast cancer (Haghi et al., 2021) found a higher prevalence of both the 14-bp deletion and the +3010, +3142 and +3187 variants in breast cancer patients compared to normal controls (Haghi et al., 2021). Recently, the association of HLA-G 3′UTR polymorphism was evaluated on various clinical parameters such as disease status, presence of disseminated tumor cells, on sHLA-G levels, and on therapy and disease outcome in non-metastatic, locally advanced breast cancer patients (Rebmann et al., 2021). The data suggest that the HLA-G 3′UTR variants +3003 C, +3187 G, and +3196 G are promising candidates for prediction of therapy and outcome in locally advanced breast cancer patients (Rebmann et al., 2021). Finally, the most recent meta-analysis, again found a close association between 3′UTR-HLA-G polymorphisms and circulating sHLA-G with breast cancer (Tizaoui et al., 2022). In conclusion, a significant association of HLA-G +3142 C/G with reduced susceptibility to cervical pathologies and high sHLA-G levels support an important role for HLA-G polymorphisms and sHLA-G expression in the pathogenesis of breast cancer.

Table 1
HLA-G gene polymorphism in breast cancer.

HLA-G gene polymorphism	Relationship with breast cancer	References
HLA-G 14-bp ins/del	14-bp insertion/deletion polymorphism in the HLA-G gene could be a genetic risk factor for the susceptibility to breast carcinoma	Eskandari-Nasab et al., 2013
HLA-G 14-bp ins/del	Patients with invasive breast ductal carcinoma (IDC) induce a higher protein expression of the HLA-G molecule	Ramos et al., 2014
HLA-G 14-bp ins/del	It is associated with reduced risk of breast cancer susceptibility based on HLA-G expression in tissues	Jeong et al., 2014
HLA-G 14-bp ins/del	HLA-G 14-bp ins/del polymorphism contributes to breast cancer susceptibility	Li et al., 2021
HLA-G 14-bp ins/del	It is a potential genetic risk factor also for progression of breast cancer	Haghi et al., 2015
HLA-G +3142 C>G	an association of HLA-G +3142 C>G polymorphism with BC susceptibility and a potential implication of elevated sHLA-G in advanced stages of BC	Zidi et al., 2006
HLA-G +3142 C>G	The +3142 C allele and +3142 C/C genotype was significantly associated with increased risk of breast cancer	Ouni et al., 2019
HLA-G 14-bp ins/del and +3142 G haplotype	Higher frequencies of HLA-G 14-bp Ins allele and Ins/+3142 G haplotype were observed in patients with breast cancer than healthy controls	Kadiam et al., 2020
14-bp deletion and the +3010, +3142 and +3187	14-bp deletion and the +3010, +3142 and +3187 variants were significantly more prevalent in breast cancer patients compared to normal controls	Haghi et al., 2015
14-bp deletion and the +3003 C, +3187 G, and +3196 G	HLA-G 3′UTR variants +3003 C, +3187 G, and +3196 G are promising candidates for the prediction of therapy and outcome in locally advanced breast cancer patients	Rebmann et al., 2021

2.5. Immunological role of HLA-G in breast cancer

The normal breast epithelial cell line MCF-12A does not express HLA-G, whereas cancer cell lines MCF-7, T47D, and MDA-MB-231 and NCI/Adr-Res have various levels of HLA-G mRNA (Elliott et al., 2011). This led to the suggestion that breast cancer cells overexpress HLA-G mRNA and protein thereby probably contributing to immune evasion (Elliott et al., 2011). Another study investigated the importance of HLA-G expression and tumor infiltrating lymphocytes (TIL) in molecular subtypes of breast cancer (Dong et al., 2012). Compared to a variety of biomarkers studied, only HLA-G expression was inversely associated with the density of TIL in breast cancer (Dong et al., 2012). Down-regulation of HLA-Ia, HLA-E, and HLA-DR and the upregulation of HLA-G and HLA-DQ are associated with immune response evasion and breast cancer aggressiveness (da Silva et al., 2013). When immunocompetent mice were injected either with syngeneic tumor cells co-expressing HLA-G5, the main soluble HLA-G isoform stabilizing conformation of human β2-microglubulin (hβ2 m), or with hβ2m+ HLA-G5- tumor cells (Loumagne et al., 2014), soluble HLA-G prevented tumor rejection. These results reinforce the importance of HLA-G to be considered as a promising target aiding in optimizing current cancer immunotherapies (Loumagne et al., 2014).

In an analysis of the content of HLA-G(+) T cells in peripheral blood from healthy women and breast cancer patients (Ostapchuk et al., 2015), the increase in the relative content of CD3(+) CD56-HLA-G(+) cells in the circulating blood in breast cancer was suggested as contributing to tumor development due to suppression of antitumor immunity (Ostapchuk et al., 2015, 2016). The MHC class II-binding peptide HLA-G26–40 was effective in eliciting tumor-reactive CD4(+) T cell responses (Ishibashi et al., 2016). Treatment with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine increased HLA-G expression in tumors and subsequently enhanced recognition by HLA-G26–40-specific T-helper lymphocytes (Ishibashi et al., 2016). When PBMCs were primed with sHLA-G1 protein prior to 48 h activation resulted in enhanced frequencies of ILT-2 expressing CD8+ T cells, and in an upregulation of immune checkpoint molecules CTLA-4, PD-1, TIM-3, and CD95 exclusively on ILT-2 positive CD8+ T cells (Schwich et al., 2020). Very recently, the nonclassical histocompatibility antigen HLA-G was found to desensitize breast cancer cells to trastuzumab by binding to NK cell receptor KIR2DL4 (Zheng et al., 2021). In addition, blockade of HLA-G/KIR2DL4 signaling made the HER2-positive breast cancer cells more sensitive to trastuzumab treatment *in vivo* (Zheng et al., 2021). The ability of HLA-G to act as an immune checkpoint protein (ICP) that is neo expressed in most tumor cells as a way to evade immune attack has been demonstrated *in vitro* as a useful target for chimeric antigen receptor (CAR)-T therapy of breast cancer (Jan et al., 2021).

Table 2
Summary of the role of HLA-G in the different tumour entities and in pregnancy.

Role of HLA-G on/ in:	Ovarian cancer	Cervical Cancer	Endometrial Cancer	Breast Cancer	Pregnancy	Malignant Melanoma
Outcome	correlation between HLA-G expression and disease progression (Jung et al., 2009)	overexpression of HLA-G leads to progression of cervical cancer (Dong et al., 2010)	correlation between HLA-G and increasing stage of endometrial cancer (Barrier et al., 2006)	HLA-G upregulation associated with shorter disease-free survival time (Kleinberg et al., 2006)	Lower HLA-G expression in miscarriage (Moreau et al., 2009; Quach et al., 2014; Ferreira et al., 2016) and preeclampsia (Goldman-Wohl et al., 2000; Yie et al., 2004).	reduced overall survival (Lin and Yan, 2018) malignant transformation and a worse prognosis with relapse or metastasis in some, but not all studies, overall effect is under debate (Marletta et al., 2021)
Immunomodulation	HLA-G expression inhibits NK-cell lyses (Kim et al., 2007)	inverse relationship between HLA-G expression and number of TILs and NK - cells (Fahim et al., 2018)		HLA-G expression inversely associated TIL in breast cancer (Dong et al., 2012)	NK cells and CD4+ T cells bind HLA-G (LeMaoult et al., 2005)	Hypoxia (Mouillot et al., 2007), HLA-G inhibits T cell proliferation and cytotoxic function. Suppression of NK cell-mediated cytolysis and induces tolerogenic DCs (Lin and Yan, 2018)
Invasiveness	Cell migration and invasion capability increased (Lin et al., 2012)	HLA-G induces genes as a key predictor of cervical cancer (Xu et al., 2022)	High HLA-G in advanced endometrial cancer compared to early-stage disease (Walentowicz-Sadlecka et al., 2019)	Soluble HLA-G related with advanced stages (Zidi et al., 2016b)	Regulation of cell migration in formation of the placenta (Le Bouteiller et al., 2007).	Increased tumour agressiveness, (Melsted et al., 2017)
Epigenetics	DNA-methylation necessary for HLA-G expression in ovarian cancer (Menendez et al., 2008)	Demethylation of HLA-G play no role in precancerous cervical lesions (Gillio-Tos et al., 2012)		Gene promoter methylation by TETs and DNMTs lead to HLA-G overexpression (Zhang et al., 2019)	Histone demethylase JHDM1D lower expression of HLA-G in PE (Luo et al., 2018)	Reduced methylation of CpG islands (Jorgensen et al., 2020)
Polymorphism	As single nucleotide polymorphisms (SNPs) regulate HLA-G expression (Schwich et al., 2019)	HLA-G 3′UTR polymorphisms related to cervical cancer development (Silva et al., 2013)		genetic risk factor for breast carcinoma (Eskandari-Nasab et al., 2013)	HLA-G gene lacks polymorphisms (LeMaoult et al., 2005)	HLA-G 5′URR polymorphism in FON+/melanoma cells (Dias et al., 2018)
Regulation	Downregulation after chemotherapy (Davidson et al., 2005)	HLA-G upregulated in HPV infected cervix and cervical carcinoma (Aggarwal et al., 2020)	Absence of HLA-G independently associated with MHC class I downregulation (Bijen et al., 2010)	enhanced by estradiol/ progesterone, (He et al., 2010) GPER triggers HLA-G (Tao et al., 2014)	HLA-G upregulates inhibitory receptors on NK cells and CD4+ T cells (LeMaoult et al., 2005).	mRNA splicing (switch from HLA-G1 to HLA-G2) (Rouas-Freiss et al., 2005) hypoxia (HIF, NF-kappaB) (Mouillot et al., 2007)
Cytokine expression	IL−1ß and TGF-ß induce HLA-G through the NF-κB (Ullah et al., 2019)	HLA-G & IL−17 stimulate progression of cervical neoplasia (Miranda et al., 2015)		stimulation with interferon (IFN)-γ (Jorgensen et al., 2020)	sHLA-G stimulates TNF-α, IFNγ, IL−10 and inhibits IL−3 (Kanai et al., 2001).	interferon-gamma (IFN-γ) (Jorgensen et al., 2020), NF-kappaB inducers, (TNF-alpha and phorbol 12-myristate 13-acetate) decreased HLA-G1 cell surface expression but increased intracytoplasmic HLA-G proteins (Zidi et al., 2006).

3. Conclusions

HLA-G (human leukocyte antigen-G) is a non-classical HLA class I molecule that was first observed in human extravillous trophoblast cells of the placenta. The expression of HLA-G and its receptors on these cells that are part of the complex machinery modulating host immune response as well as HLA-Gs presence on cancer cells clearly demonstrates its important role in immune responses. HLA-G transcripts and proteins in tumour tissues have been associated with tumorigenesis and cancer progression.

In the context of tumor biology, Paul et al. were the first to report that HLA-G expression was specifically observed in melanoma lesions, but absent in the adjacent non-tumorous tissues (Paul et al., 1998). This finding has been corroborated by numerous subsequent studies involving samples from over thirty distinct tumor types. It is now well established that HLA-G expression in cancers is closely associated with immune-suppressive microenvironments, advanced tumor stage, and poor therapeutic responses and prognosis in general (Lin and Yan, 2018; Alegre et al., 2014). HLA- G expression is associated with poor prognosis and immune evasion in both gynecological and non-gynecological cancers, indicating its potential as a biomarker and therapeutic target. The regulation of HLA-G expression in gynecological cancers, such as ovarian and cervical cancer, is influenced by several factors, including cytokines such as GM-CSF and IFN-γ, which enhance its expression, and IL-10, which can upregulate it through autocrine or paracrine signaling (Sheu and Shih, 2007). In contrast, in other cancers, such as breast cancer and malignant melanoma, HLA-G is regulated by mechanisms involving DNA methylation and the cytokine milieu (Jorgensen et al., 2020). These differences highlight the role of the tumor microenvironment and specific regulatory pathways in modulating HLA-G expression in different cancer types. For comparison, we included in Table 2 malignant melanoma as a non- gynecological tumor example.

Therefore, HLA-G appears a promising target for future antibody development as an adjunct treatment either alone or in combination with treatment strategies using immune checkpoint antibodies. A summary of the role of HLA-G in the different tumour entities and in pregnancy is shown in Table 2.

CRediT authorship contribution statement

Udo Jeschke: Writing – review & editing, Writing – original draft, Resources, Project administration, Data curation, Conceptualization. **Gernot Desoye:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Yao Ye:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources. **Julia Knabl:** Writing – original draft, Data curation, Conceptualization.

Declaration of Competing Interest

U.J. is funded by pfm medicals. All other authors declare no conflict of interest.

References

Aggarwal, R., et al., 2020. Understanding HLA-G driven journey from HPV infection to cancer cervix: adding missing pieces to the jigsaw puzzle. J. Reprod. Immunol. 142, 103205.
Alegre, E., et al., 2014. Some basic aspects of HLA-G biology. J. Immunol. Res 2014, 657625.
Alhammad, R., et al., 2020. Protein disulfide isomerase A1 regulates breast cancer cell immunorecognition in a manner dependent on redox state. Oncol. Rep. 44 (6), 2406–2418.
Alves, B.M., et al., 2015. The effect of human leukocyte antigen G alleles on human papillomavirus infection and persistence in a cohort of HIV-positive pregnant women from Brazil. Infect. Genet Evol. 34, 339–343.
Babay, W., et al., 2018. Clinicopathologic significance of HLA-G and HLA-E molecules in Tunisian patients with ovarian carcinoma. Hum. Immunol. 79 (6), 463–470.
Bainbridge, D.R., Ellis, S.A., Sargent, I.L., 1999. Little evidence of HLA-G mRNA polymorphism in Caucasian or Afro-Caribbean populations. J. Immunol. 163 (4), 2023–2027.
Bainbridge, D.R., Ellis, S.A., Sargent, I.L., 2000. The short forms of HLA-G are unlikely to play a role in pregnancy because they are not expressed at the cell surface. J. Reprod. Immunol. 47 (1), 1–16.
Barrier, B.F., et al., 2006. Characterization of human leukocyte antigen-G (HLA-G) expression in endometrial adenocarcinoma. Gynecol. Oncol. 103 (1), 25–30.
Ben Yahia, H., et al., 2018. Increased plasmatic soluble HLA-G levels in endometrial cancer. Mol. Immunol. 99, 82–86.
Ben Yahia, H., et al., 2020. Expression analysis of immune-regulatory molecules HLA-G, HLA-E and IDO in endometrial cancer. Hum. Immunol. 81 (6), 305–313.
Bijen, C.B., et al., 2010. The prognostic role of classical and nonclassical MHC class I expression in endometrial cancer. Int J. Cancer 126 (6), 1417–1427.
Bortolotti, D., et al., 2014. Implication of HLA-G 3′ untranslated region polymorphisms in human papillomavirus infection. Tissue Antigens 83 (2), 113–118.
Brenol, C.V., et al., 2012. The role of the HLA-G gene and molecule on the clinical expression of rheumatologic diseases. Rev. Bras. Reum. 52 (1), 82–91.
Burton, G.J., Jauniaux, E., Murray, A.J., 2017. Oxygen and placental development; parallels and differences with tumour biology. Placenta 56, 14–18.
Carosella, E.D., et al., 2001. HLA-G: a shield against inflammatory aggression. Trends Immunol. 22 (10), 553–555.
Castelli, E.C., et al., 2014b. Transcriptional and posttranscriptional regulations of the HLA-G gene. J. Immunol. Res 2014, 734068.
Castelli, E.C., et al., 2014a. Insights into HLA-G Genetics Provided by Worldwide Haplotype Diversity. Front Immunol. 5, 476.
Chen, H.X., et al., 2010. Upregulation of human leukocyte antigen-G expression and its clinical significance in ductal breast cancer. Hum. Immunol. 71 (9), 892–898.
Davidson, B., et al., 2005. HLA-G expression in effusions is a possible marker of tumor susceptibility to chemotherapy in ovarian carcinoma. Gynecol. Oncol. 96 (1), 42–47.
Dias, F.C., et al., 2018. The genetic diversity within the 1.4 kb HLA-G 5′ upstream regulatory region moderately impacts on cellular microenvironment responses. Sci. Rep. 8 (1), 5652.
Dong, D.D., et al., 2010. Human leukocyte antigen-G (HLA-G) expression in cervical lesions: association with cancer progression, HPV 16/18 infection, and host immune response. Reprod. Sci. 17 (8), 718–723.
Dong, D.D., et al., 2012. Importance of HLA-G expression and tumor infiltrating lymphocytes in molecular subtypes of breast cancer. Hum. Immunol. 73 (10), 998–1004.
Dunn, G.P., Old, L.J., Schreiber, R.D., 2004. The three Es of cancer immunoediting. Annu Rev. Immunol. 22, 329–360.
Elliott, R.L., et al., 2011. Human leukocyte antigen G expression in breast cancer: role in immunosuppression. Cancer Biother Radio. 26 (2), 153–157.
Ellis, S., 1990. HLA G: at the interface. Am. J. Reprod. Immunol. 23 (3), 84–86.
Eskandari-Nasab, E., et al., 2013. Association between HLA-G 3′UTR 14-bp ins/del polymorphism and susceptibility to breast cancer. Cancer Biomark. 13 (4), 253–259.
Fahim, N.M., et al., 2018. Human Leukocyte Antigen-G (HLA-G) Expression in Precancerous and Cancerous Cervical Lesions: Association with Human Papilloma Virus Infection and Host Immune Response. Egypt J. Immunol. 25 (1), 125–134.
Fainardi, E., et al., 2011. Emerging topics and new perspectives on HLA-G. Cell Mol. Life Sci. 68 (3), 433–451.
Favier, B., et al., 2010. ILT2/HLA-G interaction impairs NK-cell functions through the inhibition of the late but not the early events of the NK-cell activating synapse. FASEB J. 24 (3), 689–699.
Ferns, D.M., et al., 2016. Classical and non-classical HLA class I aberrations in primary cervical squamous- and adenocarcinomas and paired lymph node metastases. J. Immunother. Cancer 4, 78.
Ferreira, L.M., et al., 2016. A distant trophoblast-specific enhancer controls HLA-G expression at the maternal-fetal interface. Proc. Natl. Acad. Sci. USA 113 (19), 5364–5369.
Fons, P., et al., 2006. Soluble HLA-G1 inhibits angiogenesis through an apoptotic pathway and by direct binding to CD160 receptor expressed by endothelial cells. Blood 108 (8), 2608–2615.
Gan, J., et al., 2022. HLA-G 3′UTR polymorphism diplotypes and soluble HLA-G plasma levels impact cervical cancer susceptibility and prognosis. Front Immunol. 13, 1076040.
Gao, G.F., et al., 2000. Classical and nonclassical class I major histocompatibility complex molecules exhibit subtle conformational differences that affect binding to CD8alphaalpha. J. Biol. Chem. 275 (20), 15232–15238.
Gillio-Tos, A., et al., 2012. Case-control study of HLA-G promoter methylation status, HPV infection and cervical neoplasia in Curitiba, Brazil: a pilot analysis. In: BMC Cancer, 12, p. 618.
Goldman-Wohl, D.S., et al., 2000. Lack of human leukocyte antigen-G expression in extravillous trophoblasts is associated with pre-eclampsia. Mol. Hum. Reprod. 6 (1), 88–95.
Gonzalez, A., et al., 2012. The immunosuppressive molecule HLA-G and its clinical implications. Crit. Rev. Clin. Lab Sci. 49 (3), 63–84.
Guimaraes, M.C., et al., 2010. Low expression of human histocompatibility soluble leukocyte antigen-G (HLA-G5) in invasive cervical cancer with and without metastasis, associated with papilloma virus (HPV). J. Histochem Cytochem 58 (5), 405–411.
Hackmon, R., et al., 2017. Definitive class I human leukocyte antigen expression in gestational placentation: HLA-F, HLA-E, HLA-C, and HLA-G in extravillous trophoblast invasion on placentation, pregnancy, and parturition. Am. J. Reprod. Immunol. 77 (6).

Haddad, R., et al., 2011. HLA-G 14-bp insertion/deletion polymorphism is a risk factor for HTLV-1 infection. AIDS Res Hum. Retrovir. 27 (3), 283–288.
Haghi, M., et al., 2015. 14-bp Insertion/Deletion Polymorphism of the HLA-G gene in Breast Cancer among Women from North Western Iran. Asian Pac. J. Cancer Prev. 16 (14), 6155–6158.
Haghi, M., et al., 2021. Certain haplotypes of the 3′-UTR region of the HLA-G gene are linked to breast cancer. Br. J. Biomed. Sci. 78 (2), 87–91.
He, X., et al., 2010. HLA-G expression in human breast cancer: implications for diagnosis and prognosis, and effect on allocytotoxic lymphocyte response after hormone treatment in vitro. Ann. Surg. Oncol. 17 (5), 1459–1469.
Hunt, J.S., et al., 2005. HLA-G and immune tolerance in pregnancy. FASEB J. 19 (7), 681–693.
Ishibashi, K., et al., 2016. Epigenetic modification augments the immunogenicity of human leukocyte antigen G serving as a tumor antigen for T cell-based immunotherapy. Oncoimmunology 5 (6), e1169356.
Jan, C.I., et al., 2021. Targeting human leukocyte antigen G with chimeric antigen receptors of natural killer cells convert immunosuppression to ablate solid tumors. J. Immunother. Cancer 9 (10).
Jeong, S., et al., 2014. Human leukocyte antigen-G (HLA-G) polymorphism and expression in breast cancer patients. PLoS One 9 (5), e98284.
Jiang, Y., et al., 2019. Genetic variation in the HLA-G 3′UTR 14-bp insertion/deletion and the associated cancer risk: evidence from 25 case-control studies. Biosci. Rep. 39 (5).
Jorgensen, N., et al., 2020. Characterization of HLA-G Regulation and HLA Expression in Breast Cancer and Malignant Melanoma Cell Lines upon IFN-gamma Stimulation and Inhibition of DNA Methylation. Int J. Mol. Sci. 21 (12).
Jung, Y.W., et al., 2009. Correlation of human leukocyte antigen-G (HLA-G) expression and disease progression in epithelial ovarian cancer. Reprod. Sci. 16 (11), 1103–1111.
Kadiam, S., et al., 2020. Association of HLA-G 3′UTR 14-bp Ins/Del polymorphism with breast cancer among South Indian women. J. Clin. Pathol. 73 (8), 456–462.
Kanai, T., et al., 2001. Soluble HLA-G influences the release of cytokines from allogeneic peripheral blood mononuclear cells in culture. Mol. Hum. Reprod. 7 (2), 195–200.
Kapasi, K., et al., 2000. HLA-G has a concentration-dependent effect on the generation of an allo-CTL response. Immunology 101 (2), 191–200.
Kim, R., Emi, M., Tanabe, K., 2007. Cancer immunoediting from immune surveillance to immune escape. Immunology 121 (1), 1–14.
Kleinberg, L., et al., 2006. Expression of HLA-G in malignant mesothelioma and clinically aggressive breast carcinoma. Virchows Arch. 449 (1), 31–39.
Knabl, J., et al., 2023. Lower HLA-G levels in extravillous trophoblasts of human term placenta in gestational diabetes mellitus than in normal controls. Histochem Cell Biol. 159 (6), 527–535.
Konig, L., et al., 2016. The prognostic impact of soluble and vesicular HLA-G and its relationship to circulating tumor cells in neoadjuvant treated breast cancer patients. Hum. Immunol. 77 (9), 791–799.
Korkola, J.E., et al., 2003. Differentiation of lobular versus ductal breast carcinomas by expression microarray analysis. Cancer Res 63 (21), 7167–7175.
Kovats, S., et al., 1990. A class I antigen, HLA-G, expressed in human trophoblasts. Science 248 (4952), 220–223.
de Kruijf, E.M., et al., 2010. HLA-E and HLA-G expression in classical HLA class I-negative tumors is of prognostic value for clinical outcome of early breast cancer patients. J. Immunol. 185 (12), 7452–7459.
Le Bouteiller, P., et al., 1996. Placental expression of HLA class I genes. Am. J. Reprod. Immunol. 35 (3), 216–225.
Le Bouteiller, P., 2004. [The role of HLA-G expression in the embryo during implantation]. J. Gynecol. Obstet. Biol. Reprod. (Paris) 33 (1 Pt 2), S9–S12.
Le Bouteiller, P., et al., 2007. Soluble HLA-G and control of angiogenesis. J. Reprod. Immunol. 76 (1-2), 17–22.
Le Bouteiller, P., Lenfant, F., 1996. Antigen-presenting function(s) of the non-classical HLA-E, -F and -G class I molecules: the beginning of a story. Res Immunol. 147 (5), 301–313.
Le Discorde, M., et al., 2003. Expression of HLA-G in human cornea, an immune-privileged tissue. Hum. Immunol. 64 (11), 1039–1044.
Le Gal, F.A., et al., 1999. HLA-G-mediated inhibition of antigen-specific cytotoxic T lymphocytes. Int Immunol. 11 (8), 1351–1356.
Lefebvre, S., et al., 2000. Modulation of HLA-G expression in human thymic and amniotic epithelial cells. Hum. Immunol. 61 (11), 1095–1101.
Lefebvre, S., et al., 2002. Specific activation of the non-classical class I histocompatibility HLA-G antigen and expression of the ILT2 inhibitory receptor in human breast cancer. J. Pathol. 196 (3), 266–274.
LeMaoult, J., et al., 2005. HLA-G up-regulates ILT2, ILT3, ILT4, and KIR2DL4 in antigen presenting cells, NK cells, and T cells. FASEB J. 19 (6), 662–664.
Li, P., et al., 2021. HLA-G/sHLA-G and HLA-G-Bearing Extracellular Vesicles in Cancers: Potential Role as Biomarkers. Front Immunol. 12, 791535.
Li, T., et al., 2015. Genetic polymorphism in HLA-G 3′UTR 14-bp ins/del and risk of cancer: a meta-analysis of case-control study. Mol. Genet Genom. 290 (4), 1235–1245.
Li, X.J., et al., 2012. Human leukocyte antigen-G (HLA-G) expression in cervical cancer lesions is associated with disease progression. Hum. Immunol. 73 (9), 946–949.
Lin, A., et al., 2012. HLA-G expression is associated with metastasis and poor survival in the Balb/c nu/nu murine tumor model with ovarian cancer. Int J. Cancer 131 (1), 150–157.
Lin, A., et al., 2013. Multiple steps of HLA-G in ovarian carcinoma metastasis: alter NK cytotoxicity and induce matrix metalloproteinase-15 (MMP-15) expression. Hum. Immunol. 74 (4), 439–446.
Lin, A., Yan, W.H., 2018. Heterogeneity of HLA-G Expression in Cancers: Facing the Challenges. Front Immunol. 9, 2164.
López-Botet, M., Bellón, T., 1999. Natural killer cell activation and inhibition by receptors for MHC class I. Curr. Opin. Immunol. 11 (3), 301–307.
Loumagne, L., et al., 2014. In vivo evidence that secretion of HLA-G by immunogenic tumor cells allows their evasion from immunosurveillance. Int J. Cancer 135 (9), 2107–2117.
Loustau, M., et al., 2020. HLA-G Neo-Expression on Tumors. Front Immunol. 11, 1685.
Luo, S., et al., 2018. Decreased expression of JHDMID in placenta is associated with preeclampsia through HLA-G. J. Hum. Hypertens. 32 (6), 448–454.
Maejima, M., et al., 1997. Presence of HLA-G-expressing cells modulates the ability of peripheral blood mononuclear cells to release cytokines. Am. J. Reprod. Immunol. 38 (2), 79–82.
Manaster, I., et al., 2012. MiRNA-mediated control of HLA-G expression and function. PLoS One 7 (3), e33395.
Marletta, S., et al., 2021. HLA-G expression in melanomas. Int Rev. Immunol. 40 (5), 330–343.
Medeiros, F.S., et al., 2018. Variation sites at the HLA-G 3′ untranslated region confer differential susceptibility to HIV/HPV co-infection and aneuploidy in cervical cell. PLoS One 13 (10), e0204679.
Melsted, W.N., et al., 2017. HLA class Ia and Ib molecules and FOXP3+ TILs in relation to the prognosis of malignant melanoma patients. Clin. Immunol. 183, 191–197.
Menendez, L., et al., 2008. Epigenetic changes within the promoter region of the HLA-G gene in ovarian tumors. Mol. Cancer 7, 43.
Menier, C., et al., 2002. MICA triggering signal for NK cell tumor lysis is counteracted by HLA-G1-mediated inhibitory signal. Int J. Cancer 100 (1), 63–70.
Menier, C., et al., 2004. Erythroblasts secrete the nonclassical HLA-G molecule from primitive to definitive hematopoiesis. Blood 104 (10), 3153–3160.
Menier, C., et al., 2009. Human leukocyte antigen-G is expressed in advanced-stage ovarian carcinoma of high-grade histology. Hum. Immunol. 70 (12), 1006–1009.
Metcalfe, S., et al., 2013. The association between human leukocyte antigen (HLA)-G polymorphisms and human papillomavirus (HPV) infection in Inuit women of northern Quebec. Hum. Immunol. 74 (12), 1610–1615.
Miranda, L.N., et al., 2015. Greater expression of the human leukocyte antigen-G (HLA-G) and interleukin-17 (IL-17) in cervical intraepithelial neoplasia: analytical cross-sectional study. Sao Paulo Med J. 133 (4), 336–342.
Mociornita, A.G., et al., 2013. Can HLA-G polymorphisms predict the development of cardiac allograft vasculopathy? Hum. Immunol. 74 (4), 464–467.
Morandi, F., et al., 2010. A novel mechanism of soluble HLA-G mediated immune modulation: downregulation of T cell chemokine receptor expression and impairment of chemotaxis. PLoS One 5 (7), e11763.
Moreau, P., et al., 1999. IL-10 selectively induces HLA-G expression in human trophoblasts and monocytes. Int Immunol. 11 (5), 803–811.
Moreau, P., Flajollet, S., Carosella, E.D., 2009. Non-classical transcriptional regulation of HLA-G: an update. J. Cell Mol. Med 13 (9b), 2973–2989.
Mouillot, G., et al., 2007. Hypoxia modulates HLA-G gene expression in tumor cells. Hum. Immunol. 68 (4), 277–285.
Muz, B., et al., 2015. Hypoxia Promotes Dissemination and Colonization in New Bone Marrow Niches in Waldenström Macroglobulinemia. Mol. Cancer Res. 13 (2), 263–272.
Naji, A., et al., 2007. Soluble HLA-G and HLA-G1 expressing antigen-presenting cells inhibit T-cell alloproliferation through ILT-2/ILT-4/FasL-mediated pathways. Hum. Immunol. 68 (4), 233–239.
Nazari, N., Farjadian, S., 2016. Natural Killer Cell Cytotoxicity Against SKOV3 after HLA-G Downregulation by shRNA. Iran. J. Immunol. 13 (3), 178–185.
O'Callaghan, C.A., Bell, J.I., 1998. Structure and function of the human MHC class Ib molecules HLA-E, HLA-F and HLA-G. Immunol. Rev. 163, 129–138.
Ostapchuk, E.O., et al., 2015. Content of HLA-G(+) T Cells in the peripheral blood from healthy women and breast cancer patients. Bull. Exp. Biol. Med 159 (5), 649–651.
Ostapchuk, E.O., et al., 2016. Erratum to: Content of HLA-G(+) T Cells in the Peripheral Blood from Healthy Women and Breast Cancer Patients. Bull. Exp. Biol. Med 160 (4), 592.
Ouni, N., et al., 2019. The Impact of HLA-G 3′UTR Polymorphisms in Breast Cancer in a Tunisian Population. Immunol. Invest 48 (5), 521–532.
Pangault, C., et al., 1999. HLA-G protein expression is not induced during malignant transformation. Tissue Antigens 53 (4 Pt 1), 335–346.
Paul, P., et al., 1998. HLA-G expression in melanoma: a way for tumor cells to escape from immunosurveillance. Proc. Natl. Acad. Sci. USA 95 (8), 4510–4515.
Paul, P., et al., 1999. Heterogeneity of HLA-G gene transcription and protein expression in malignant melanoma biopsies. Cancer Res 59 (8), 1954–1960.
Persson, G., et al., 2017. HLA class Ib in pregnancy and pregnancy-related disorders. Immunogenetics 69 (8-9), 581–595.
Ponte, M., et al., 1999. Inhibitory receptors sensing HLA-G1 molecules in pregnancy: decidua-associated natural killer cells express LIR-1 and CD94/NKG2A and acquire p49, an HLA-G1-specific receptor. Proc. Natl. Acad. Sci. USA 96 (10), 5674–5679.
Provatopoulou, X., et al., 2012. Soluble human leukocyte antigen-G expression in patients with ductal and lobular breast malignancy. Anticancer Res 32 (3), 1021–1026.
Quach, K., et al., 2014. A combination of single nucleotide polymorphisms in the 3′untranslated region of HLA-G is associated with preeclampsia. Hum. Immunol. 75 (12), 1163–1170.
Rajagopalan, S., Long, E.O., 1999. A human histocompatibility leukocyte antigen (HLA)-G-specific receptor expressed on all natural killer cells. J. Exp. Med 189 (7), 1093–1100.
Ramos, C.S., et al., 2014. Analysis of HLA-G gene polymorphism and protein expression in invasive breast ductal carcinoma. Hum. Immunol. 75 (7), 667–672.

Rebmann, V., et al., 2003. Secretion of sHLA-G molecules in malignancies. Semin Cancer Biol. 13 (5), 371–377.
Rebmann, V., et al., 2014. HLA-G as a tolerogenic molecule in transplantation and pregnancy. J. Immunol. Res 2014, 297073.
Rebmann, V., et al., 2021. Systematic Evaluation of HLA-G 3′Untranslated Region Variants in Locally Advanced, Non-Metastatic Breast Cancer Patients: UTR-1, 2 or UTR-4 are Predictors for Therapy and Disease Outcome. Front Immunol. 12, 817132.
Ristich, V., et al., 2005. Tolerization of dendritic cells by HLA-G. Eur. J. Immunol. 35 (4), 1133–1142.
Rolfsen, G.B., et al., 2014. HLA-G polymorphism and breast cancer. Int J. Immunogenet. 41 (2), 143–148.
Rouas-Freiss, N., et al., 1999. The immunotolerance role of HLA-G. Semin Cancer Biol. 9 (1), 3–12.
Rouas-Freiss, N., et al., 2005. Switch of HLA-G alternative splicing in a melanoma cell line causes loss of HLA-G1 expression and sensitivity to NK lysis. Int J. Cancer 117 (1), 114–122.
Roussev, R.G., Coulam, C.B., 2007. HLA-G and its role in implantation (review). J. Assist Reprod. Genet 24 (7), 288–295.
Rutten, M.J., et al., 2014. HLA-G expression is an independent predictor for improved survival in high grade ovarian carcinomas. J. Immunol. Res 2014, 274584.
Sato, M., et al., 2017. Regeneration of cervical reserve cell-like cells from human induced pluripotent stem cells (iPSCs): A new approach to finding targets for cervical cancer stem cell treatment. Oncotarget 8 (25), 40935–40945.
Sayed, D., et al., 2010. HLA-G and its relation to proliferation index in detection and monitoring breast cancer patients. Tissue Antigens 75 (1), 40–47.
Schwich, E., et al., 2019. HLA-G 3′ untranslated region variants +3187G/G, +3196G/G and +3035T define diametrical clinical status and disease outcome in epithelial ovarian cancer. Sci. Rep. 9 (1), 5407.
Schwich, E., et al., 2020. Soluble HLA-G and HLA-G Bearing Extracellular Vesicles Affect ILT-2 Positive and ILT-2 Negative CD8 T Cells Complementary. Front Immunol. 11, 2046.
Sheu, J.J., Shih, M., 2007. Ie, *Clinical and biological significance of HLA-G expression in ovarian cancer.* Semin Cancer Biol. 17 (6), 436–443.
Shiroishi, M., et al., 2003. Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially to HLA-G. Proc. Natl. Acad. Sci. USA 100 (15), 8856–8861.
da Silva, G.B., et al., 2013. Expression of the Classical and Nonclassical HLA Molecules in Breast Cancer. Int J. Breast Cancer 2013, 250435.
Silva, I.D., et al., 2013. HLA-G 3′UTR polymorphisms in high grade and invasive cervico-vaginal cancer. Hum. Immunol. 74 (4), 452–458.
da Silva, N.C.H., et al., 2023. Contribution of HLA-G and FOXP3 genes and proteins in the severity of cervical intraepithelial neoplasia during HPV infection. Hum. Immunol. 84 (8), 408–417.
Simões, R.T., et al., 2009. HLA-G polymorphisms in women with squamous intraepithelial lesions harboring human papillomavirus. Mod. Pathol. 22 (8), 1075–1082.
Singer, G., et al., 2003. HLA-G is a potential tumor marker in malignant ascites. Clin. Cancer Res 9 (12), 4460–4464.
Solier, C., et al., 2001. HLA-G unique promoter region: functional implications. Immunogenetics 53 (8), 617–625.
Sun, J., et al., 2016. Long non-coding RNA HOTAIR modulates HLA-G expression by absorbing miR-148a in human cervical cancer. Int J. Oncol. 49 (3), 943–952.
Svendsen, S.G., et al., 2013. The expression and functional activity of membrane-bound human leukocyte antigen-G1 are influenced by the 3′-untranslated region. Hum. Immunol. 74 (7), 818–827.
Tao, S., et al., 2014. GPER mediated estradiol reduces miR-148a to promote HLA-G expression in breast cancer. Biochem Biophys. Res Commun. 451 (1), 74–78.
Tizaoui, K., et al., 2022. 3'UTR-HLA-G polymorphisms and circulating sHLA-G are associated with breast cancer: Evidence from a meta-analysis. Immunol. Lett. 248, 78–89.
Torres, M.P., et al., 2009. Immunopathogenesis of ovarian cancer. Minerva Med 100 (5), 385–400.
Turashvili, G., et al., 2005. Differentiation of tumours of ductal and lobular origin: II. Genomics of invasive ductal and lobular breast carcinomas. Biomed. Pap. Med Fac. Univ. Palacky. Olomouc Czech Repub. 149 (1), 63–68.
Ueshima, C., et al., 2015. The Killer Cell Ig-like Receptor 2DL4 Expression in Human Mast Cells and Its Potential Role in Breast Cancer Invasion. Cancer Immunol. Res 3 (8), 871–880.
Ullah, M., et al., 2019. High Expression of HLA-G in Ovarian Carcinomatosis: The Role of Interleukin-1beta. Neoplasia 21 (3), 331–342.
Viac, J., et al., 1993. Expression of immune associated surface antigens of keratinocytes in human papillomavirus-derived lesions. Immunobiology 188 (4-5), 392–402.
Walentowicz-Sadlecka, M., et al., 2019. The analysis of human leukocyte antigen-G level in patients with endometrial cancer by Western blot technique. Am. J. Reprod. Immunol. 81 (1), e13070.
Wuerfel, F.M., et al., 2020. HLA-G and HLA-F protein isoform expression in breast cancer patients receiving neoadjuvant treatment. Sci. Rep. 10 (1), 15750.
Xu, H.H., et al., 2018. Association of HLA-G 3′ UTR polymorphism and expression with the progression of cervical lesions in human papillomavirus 18 infections. Infect. Agent Cancer 13, 42.
Xu, H.H., et al., 2022. A Novel Prognostic Risk Model for Cervical Cancer Based on Immune Checkpoint HLA-G-Driven Differentially Expressed Genes. Front Immunol. 13, 851622.
Xu, H.H., et al., 2023. Dynamic changes of soluble HLA-G and cytokine plasma levels in cervical cancer patients: potential role in cancer progression and immunotherapy. J. Cancer Res Clin. Oncol. 149 (8), 4195–4204.
Yan, W.H., Fan, L.A., 2005. Residues Met76 and Gln79 in HLA-G alpha1 domain involve in KIR2DL4 recognition. Cell Res 15 (3), 176–182.
Yang, Y.C., et al., 2014. Human leucocyte antigen-G polymorphisms are associated with cervical squamous cell carcinoma risk in Taiwanese women. Eur. J. Cancer 50 (2), 469–474.
Yie, S.M., et al., 2004. HLA-G protein concentrations in maternal serum and placental tissue are decreased in preeclampsia. Am. J. Obstet. Gynecol. 191 (2), 525–529.
Yie, S.M., Xiao, R., Librach, C.L., 2006. Progesterone regulates HLA-G gene expression through a novel progesterone response element. Hum. Reprod. 21 (10), 2538–2544.
Zhang, D., et al., 2019. Role of gene promoter methylation regulated by TETs and DNMTs in the overexpression of HLA-G in MCF-7 cells. Exp. Ther. Med 17 (6), 4709–4714.
Zhang, W., et al., 2008. Human inhibitory receptor immunoglobulin-like transcript 2 amplifies CD11b+Gr1+ myeloid-derived suppressor cells that promote long-term survival of allografts. Transplantation 86 (8), 1125–1134.
Zhang, X., et al., 2016. Lesion HLA-G5/-G6 isoforms expression in patients with ovarian cancer. Hum. Immunol. 77 (9), 780–784.
Zheng, G., et al., 2021. Interaction between HLA-G and NK cell receptor KIR2DL4 orchestrates HER2-positive breast cancer resistance to trastuzumab. Signal Transduct. Target Ther. 6 (1), 236.
Zheng, N., et al., 2011. Up-regulation of HLA-G expression in cervical premalignant and malignant lesions. Tissue Antigens 77 (3), 218–224.
Zhou, J.H., et al., 2006. Altered expression of cellular membrane molecules of HLA-DR, HLA-G and CD99 in cervical intraepithelial neoplasias and invasive squamous cell carcinoma. Life Sci. 78 (22), 2643–2649.
Zidi, I., et al., 2006. Increase in HLA-G1 proteolytic shedding by tumor cells: a regulatory pathway controlled by NF-kappaB inducers. Cell Mol. Life Sci. 63 (22), 2669–2681.
Zidi, I., et al., 2016b. Association of HLA-G +3142C>G polymorphism and breast cancer in Tunisian population. Immunol. Res 64 (4), 961–968.
Zidi, I., et al., 2016a. Pregnancy and breastfeeding: a new theory for sHLA-G in breast cancer patients? Immunol. Res 64 (2), 636–639.