HLA-G/sHLA-G and HLA-G-Bearing Extracellular Vesicles in Cancers: Potential Role as Biomarkers

Peilong Li1,2,3†, Nan Wang4†, Yi Zhang5, Chuanxin Wang1,2,3* and Lutao Du1,2,3*

1 Department of Clinical Laboratory, The Second Hospital, Cheloo College of Medicine, Shandong University, Jinan, China, 2 Shandong Engineering & Technology Research Center for Tumor Marker Detection, Jinan, China, 3 Shandong Provincial Clinical Medicine Research Center for Clinical Laboratory, Jinan, China, 4 School of Public Health, Shandong First Medical University & Shandong Academy of Medical Sciences, Taian, China, 5 Department of Respiratory and Critical Care Medicine, Qilu Hospital, Cheloo College of Medicine, Shandong University, Jinan, China

As a non-classic major histocompatibility complex (MHC) class I molecule, human leukocyte antigen G (HLA-G) is expressed in fetal-maternal interface and immunopriviliged site only in healthy condition, and in pathological conditions such as cancer, it can be de novo expressed. It is now widely accepted that HLA-G is a key molecule in the process of immune escape of cancer cells, which is ubiquitously expressed in the tumor environment. This raises the possibility that it may play an adverse role in tumor immunity. The expression level of HLA-G has been demonstrated to be highly correlated with clinical parameters in many tumors, and its potential significance in the diagnosis and prognosis of cancer has been postulated. However, because HLA-G itself has up to seven different subtypes, and for some subtypes, detected antibodies are few or absent, it is hard to evaluate the actual expression of HLA-G in tumors. In the present work, we described (a) the structure and three main forms of HLA-G, (b) summarized the mechanism of HLA-G in the immune escape of tumor cells, (c) discussed the potential role of HLA-G as a tumor marker, and reviewed (d) the methods for detecting and quantifying HLA-G.

Keywords: HLA-G, tumor, immune escape, extracellular vesicles, biomarker

INTRODUCTION

As early as 1983, human leukocyte antigen G (HLA-G) is first observed on the cytotrophoblast at the fetal-maternal interface (1). As a class of major histocompatibility complex (MHC) I molecules, HLA-G showed low polymorphic in the coding region, while several polymorphism have been describe in non-coding region of the locus (3’UTR, and 5’URR regions) (2). The exons and introns of the HLA-G gene are the same as those of classic MHC class molecules, consisting of eight exons and seven introns (3, 4). However, HLA-G shows only limited genetic polymorphism. The main reason can be attributed to that the terminator of HLA-G is located in the second codon of exon 6, and thus most of exon 6 and all of exons 7 and 8 cannot be translated into protein (5, 6). HLA-G can exist in a variety of structures, which can not only be expressed on the cell surface but also exist in
the form of secretion (7, 8). There are seven isoforms, which encode four membrane-bound (HLA-G1, -G2, -G3, and -G4) and three soluble (HLA-G5, -G6, and -G7) protein isoforms (9). Each HLA-G subtype contains one to three spherical domains (α1, α2, and α3) encoded by exon 2, exon 3, and exon 4 (Figure 1).

HLA-G is initially considered to be useful for fetal establishment and maintenance of maternal-fetal immune tolerance (10, 11). Therefore, initial studies have mainly focused on its role in regulating maternal immune cell responses and protecting the fetus from natural killer (NK) cell-mediated lysis (12–14). Fetal and tumor development are similar and characterized by rapid tissue proliferation, which is associated with the expressions of anti-apoptotic factors and telomerase (14–17). It is worth noting that since immunoregulatory sites can be shared between tumor development and placenta formation, both placenta and tumor are protected by the immune system and resistant to induction by the immune microenvironment. Therefore, the research focus of HLA-G has gradually shifted to tumors (18).

In the malignant environment where tumors occur, the expression of HLA-G in melanoma is first reported in 1998 (19), and then the abnormal expression of HLA-G is observed in a variety of malignant tumors, such as lung cancer (20–28), gastric cancer (29–37), ovarian cancer (OC) (38–47), breast cancer (48–54), and hematopoietic tumor (55–58). With the deepening of research, the expression of HLA-G in solid malignant tumors and its potential clinical relevance have attracted increasing attention (59). Abnormal expression of HLA-G plays a variety of roles in the progression of malignant tumors, such as inducing apoptosis, inhibiting immune cytolysis and cytotoxicity, and chemotaxis of regulatory cells and damage of different immune effector cells through receptor binding and/or trogocytosis (60).

In addition, HLA-G is secreted in a variety of body fluids, as either free soluble HLA-G (sHLA-G) or part of extracellular vesicles (EVs), and it has been extensively studied as tumor markers. Among them, sHLA-G is derived from the secretion of sHLA-G homotypes, such as HLA-G5, HLA-G6, and HLA-G7, and the shedding of membrane-bound HLA-G homotypes, such as HLA-G1, cleaved by proteolytic hydrolysis (61). Soluble isoforms can be detected in saliva (62, 63), ascitic fluid (47), plasma (26, 33, 48, 49, 56, 64–69), thymus (70), seminal plasma (71), cerebrospinal fluid (CSF) (72, 73), human first trimester and term placenta in situ and in vitro (74), and cell culture supernatant (75, 76). High levels of sHLA-G are correlated with tumor histological type, lymph node metastasis, and patient survival, which can be used as a tumor marker to provide a basis for early diagnosis, differentiation, and prognosis (21). However, it remains largely unclear whether HLA-G-bearing EVs are produced by tumor cells, while there is a functional association between the HLA-G-bearing EVs and various tumors, such as melanoma, breast cancer, and kidney cancer (77). In summary, sHLA-G and HLA-G-bearing EVs may provide unpredictable diagnostic opportunities to monitor tumor status and progression.

**FIGURE 1** | HLA-G protein isoforms and immune-inhibitory function.
HLA-G: A KEY IMMUNE EVASION MOLECULE IN TUMORS

In cancer, abnormal expression of HLA-G is considered a key strategy of tumor cells to evade immune surveillance, which is strongly supported by the high incidence of tumors in patients treated with immunosuppressive agents after organ and stem cell transplantation (78). The continued construction of tumor phenotypes is thought to be a result of immune-mediated tumor recognition, a phenomenon known as immune editing of cancer. Three stages define the process of immune editing: elimination (immune surveillance), balance (duration/dormancy), and escape (progression) (79, 80). These three stages integrate the immune system’s ability to protect the host from cancer and promote cancer development (81). HLA-G is involved in all three stages, and it is highly necessary to understand the role of HLA-G in tumor immune escape to better develop effective anti-tumor strategies. In the present work, we summarized the main mechanisms of HLA-G-mediated immunosuppression in three aspects as follows:

1. Inhibitory receptors of HLA-G, such as KIR2DL4/CD158d, ILT-2/CD85j, ILT-4/CD85d, CD8, and CD160, can directly exert the immunosuppressive effect by HLA-G. These inhibitory receptors can express in all monocytes, as well as B cells, T cells, and NK cells. Especially, ILT2 receptors are present in subgroups of dendritic cells (DCs) and myeloid-derived suppressive cells (MDSCs) (82–85). ILT4 receptors are mainly expressed in DCs, neutrophils, monocytes, and MDSCs (86–88). KIR2DL4 receptors are mainly expressed in decidual NK cells (89). The mechanisms by which these receptors participate in immunosuppressive effects induced by HLA-G include inhibition of differentiation, cytokine secretion and chemotaxis, immune cell proliferation, cytotoxicity, and induction of MDSCs or M2-type macrophages and regulatory cells (90, 91) (Figure 1).

2. MDSCs, regulatory T cells (Tregs), and tolerogenic DCs can participate in immune escape regulated by HLA-G via an indirect immunosuppressive way. The sub-population of tolerogenic DCs, DC-10s, expresses a high level of HLA-G and induces adaptive type 1 Tregs (Tr1) through the HLA-G/ILT4 signaling pathway (92). On the other hand, tolerogenic DCs produce CD8+CD28+ and CD4+CD25+CTLA-4+ Tregs under the induction of HLA-G, which further strengthens the ability of immune escape of tumor cells (93).

3. HLA-G uses dynamic transfer mechanisms between cells, such as trogocytosis (membrane-bound HLA-G) and EVs (membrane-bounded and sHLA-G) (77, 94–96) (Figure 2). Trogocytosis is the process of transporting secretory vesicles or other membrane vesicles from the cell through the cell membrane (97). Activated T cells and NK cells obtain membrane fragments containing functional HLA-G from HLA-G+ or tumor cells through the process of exocytosis. HLA-G-modified cells can immediately reverse immune

**FIGURE 2** | HLA-G-bearing EVs with potential immunological and clinical relevance. NK cells and T cells acquire HLA-G from HLA-G+tumor cells or HLA-G+immune cells via the process of trogocytosis and/or EVs.
effector term EVs are phospholipid bilayer-enclosed vesicles, which are highly heterogeneous in size, molecular content, and membrane composition depending on the state, micro-environment, and the cell of origin (100, 101). According to biogenesis, EVs are characterized by apoptotic bodies (AB) (>500 nm), microvesicles (100-1,000 nm), and exosomes (70-150 nm) (102). In the tumor state, especially in the established acidic microenvironment, EVs can directly fuse with cancer cells, or carry out the transportation and exchange of biologically active substances through endocytosis, phagocytosis, and micropinocytosis, thereby contributing to the intercellular signaling mechanism, providing the tumor with oxygen, metabolites, nutrients and other soluble factors, and making tumor development possible (103).

ELEVATED HLA-G IN TUMOR PATIENT TISSUES AS TUMOR INDICATORS

Over the years, many studies have reported that HLA-G is preferentially detected in the primary tumor site and metastatic tumor site, while it is rarely detected in the tumor spontaneous regression site, adjacent tissues, or healthy tissues (104–106). Immunohistochemical (IHC) staining is usually used to detect the expression frequency of HLA-G in tissues, which is combined with clinical results for analysis. In most cancers, the expression of HLA-G is related to the patient’s poor clinical outcome (25, 107, 108).

In liver tissue, HLA-G is detected in the primary site of hepatocellular carcinoma, while its expression is low in benign lesions represented by liver cirrhosis (109). In thyroid tissue, the cell staining efficiency of HLA-G antigen in follicular adenocarcinoma and thyroid cancer is significantly higher compared with the normal thyroid and goiter (110). In ovarian tissue, HLA-G is always co-localized with CA125 protein, indicating that OC cells express HLA-G, while normal cells do not (39). In cervical cancer lesions, the expression of HLA G is an important predictor of CIN I and age, which is not affected by other variables. In addition, HLA-G interacts with immunosuppression induced by human papillomavirus infection, leading to more serious clinical outcomes observed in patients with CIN III and invasive cervical cancer (111, 112).

In oral squamous cell carcinoma (OSCC), according to IHC and reverse transcription-polymerase chain reaction (RT-PCR) results, the higher the TNM stage, the higher the protein expression level of HLA-G, and the histological grade and lymph node metastasis are positively correlated with the expression of HLA-G (107). These results indicate that HLA-G is related to the malignant transformation of tumors, supporting that HLA-G is an indicator of early diagnosis and dynamic monitor. The expression of HLA-G in tissues may also be an important indicator for the prognosis of cancer patients. In 201 colon cancer patients, IHC staining shows that the survival time of patients with HLA-G-positive tumors is significantly shorter compared with those carrying HLA-G-negative tumors (113). In multivariate analysis, HLA-G shows the potential as an independent prognostic factor.

In a joint analysis of HLA class I, HLA-E, and HLA-G to predict the prognosis of colorectal cancer (CRC), three tumor immune phenotypes are generated by comprehensively analyzing the expressions of all markers, resulting in strong immune system tumor recognition, intermediate immune system tumor recognition, and poor immune system tumor recognition. These immune phenotypes represent important and independent clinical prognostic characteristics of colon cancer (108). In nasopharyngeal carcinoma (NPC), HLA-G is positively correlated with CD68+ macrophages and IL-10 expression, indicating that HLA-G may regulate immune escape in NPC (114). Among 522 NPC specimens, the expression of HLA-G at the protein level is detected in 79.2% of cases. In addition, the high expression of HLA-G predicts the low survival rate of NPC patients (114). Moreover, HLA-G is suggested to be an independent predictor of cancers, such as esophageal squamous cell carcinoma (115), gastric cancer (37, 116), breast cancer (52, 117, 118), and OC (38, 44, 119). Finally, the differential expressions of HLA-G can also help predict and diagnose different subtypes. Significant differences between groups have been observed between low-grade glioma and high-grade glioma tissues (120). Besides, the expression of HLA-G in the non-luminal subtypes of invasive ductal carcinoma of the breast is significantly higher compared with the luminal subtypes (121).

SHLA-G IN THE BLOOD OF TUMOR PATIENTS AS CIRCULATING TUMOR MARKERS

In the serum of healthy people, the content of HLAG is 20 ng/mL and significantly lower compared with cancer patients. sHLA-G is produced and secreted mainly by immune cells and tumors (122). For example, in acute leukemia, the level of sHLA-G in T cells and monocytes in the serum is detected by enzyme-linked immunosorbent assay (ELISA), which is averagely five times higher compared with healthy controls. Moreover, sHLA-G can be secreted in vitro by DCs, lymphocytes, plasma cells, and monocytes/macrophages (56), and these secreted sHLA-G molecules cause anti-tumor reactions locally in the tumor or along with the circulatory system to the whole body. Next, we described the current status of sHLA-G in the serum of tumor patients as tumor markers from the aspects of diagnosis, prognosis, and identification (Table 1).

In terms of diagnosis, sHLA-G is abnormally expressed in the plasma in breast ductal carcinoma (131), head and neck squamous cell carcinoma (129), gastric cancer (33), CRC (125), and papillary thyroid carcinoma (65), which is considered to be a preoperative diagnosis of cancer histopathology potential marker of aggressiveness. It is worth noting that in gastric cancer, researchers have found that sHLA-G in combination with common serum tumor markers, such as CA72-4, CA125,
# TABLE 1 | Clinical research involving diagnosis and prognosis of sHLA-G in the blood.

| Cancer type                  | Sample source | Sample size | Methods (Ab) | Experimental result evaluation                                                                 | Expression evaluation of HLA-G                                                                 | Ref. |
|-----------------------------|---------------|-------------|--------------|---------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|------|
| Breast cancer               | plasma 142    | 154 (after  |
|                            |               | NACT)       | ELISA        | The total concentration of sHLA-G plasma levels (median [range] ng/mL) from BC patients (n = 102) before (41.3 [4.4–117.6]) and after (44.6 [3.1–117.6]) NACT was significantly increased compared with 16 healthy female controls (16.3 [4.0–37.6]). | The free soluble and vesicular HLA-G as prognostic markers, whereas the total sHLA-G levels without dividing into subcomponents were not related to clinical outcome. | (48) |
|                            | plasma 92     | (patients)  | ELISA        | Concentration of the plasma sHLA-G was with the median of 82.19 U/mL (range 13.50 –191.37) for BC patients, and 9.65 U/mL (range 4.38 – 69.69) for normal controls. | Plasma sHLA-G levels might be a useful preoperative biomarker for diagnosis. | (49) |
| Ovarian cancer              | plasma 79     | (patients)  | ELISA        | In OC patients, sHLA-G1 levels were more increased than HLA-G5 levels. | As a potential biomarker for advanced and complicated OC. | (66) |
| Lung cancer                 | plasma 137    | (patients)  | ELISA        | In lung cancer patients, the plasma levels (median [range]) of sHLA-G were significantly increased compared with healthy controls (34 ng/mL [3.6–160] vs. 14 ng/mL [1.9–96]). | Plasma levels of sHLA-G is potent predictors for overall survival (OS) in lung cancer patients. | (26) |
|                            | plasma 91(patients) 150 (controls) | ELISA | The median plasma sHLA-G was 34.0 U/mL (range 3.13 – 275.5) in NSCLC patients and 20.4 U/mL (range 0.97 -270.6) in controls. | HLA-G may be a potential therapeutic target, and plasma sHLA-G of NSCLC patients can be used as a prognostic factor for NSCLC. | (23) |
| Colorectal cancer           | plasma 133    | (patients)  | ELISA        | sHLA-G levels were higher in patients with mucinous carcinoma (MC). | A useful prognostic marker and predictive biomarker of therapeutic response in advanced CRC. | (64) |
| Endometrial cancer          | plasma 40     | (patients)  | ELISA        | The majority of EC patients expressed the sHLA-G1 subtype (75%), and only 25% expressed the HLA-G5 isoforms. | Related to clinical progress. | (123) |
| Thyroid carcinoma           | plasma 85     | (patients)  | ELISA        | sHLA-G was decreased in patients with invasion. | Associated with tumor invasion. | (65) |
|                            |               | 77 (30 days after surgery) | ELISA (MEM-G/9) | | | |
| Thyroid carcinoma           | plasma 121    | (patients)  | ELISA        | sHLA-G level was significantly higher in PTC patients than those without markers of aggressiveness | sHLA-G as a potential novel marker of PTC aggressiveness | (67) |
|                            |               | 150 (patients) | (underwent PTC surgery) | ELISA (MEM-G/9) | | |
| Esophageal squamous cell carcinoma | plasma 41     | (patients)  | ELISA        | The median plasma concentration of sHLA-g in ESCC patients was 152.4 U/mL (range 28.8-239.5) versus 8.9 U/mL (range 4.6-63.5) in normal controls. | May be a useful biomarker for preoperative diagnosis. | (115) |
|                            |               | 153 (controls) | ELISA | Levels of sHLA-G were higher in the breast cancer group (median117.2 U/mL) compared with the control group (median 10.1 U/mL; P = 0.001). | Measurement of sHLA-G concentrations has diagnostic value for detecting breast cancer and metastasis. | (124) |
| Lung cancer                 | serum 80      | (patients)  | ELISA        | The mean serum level of sHLA-G in NSCLC patients (53.3 ± 4.6 U/mL) was significantly increased compared with controls (8.36 ± 0.4 U/mL). | Serum sHLA-G levels in NSCLC patients could be useful biomarkers for the diagnostic and prognosis of NSCLC. | (22) |
|                            | serum 80      | (controls)  | ELISA        | | | |
| Colorectal cancer           | serum 398     | (patients)  | ELISA (MEM-G/9) | Median sHLA-G was significantly higher in cancer compared with normal CRC, hyperplastic polyps, inflammatory bowel disease, and adenomas (all P < 0.001). | May be a useful indicator in differentiating colorectal cancer from benign colorectal diseases. | (125) |
| Thyroid carcinoma Oral squamous cell carcinoma | serum 145 | (patients)  | ELISA (MEM-G/9) | shHLA-g in serum was increased in patients with thyroid carcinoma compared with healthy controls (P < 0.05). | Affects the progression of thyroid cancer. | (126) |
| Head and neck squamous      | venous 383    | (patients)  | PCR          | Individuals with Del/Ins and Ins/Ins genotypes were at greater risk of HNSCC disease than those with Del/Del genotypes. | The C/C, Del/Ins and Ins/Ins genotypes as well as C and Ins alleles may be the major | (128) |
TABLE 1 | Continued

| Cancer type        | Sample source | Sample size | Methods (Ab) | Experimental result evaluation                                                                 | Expression evaluation of HLA-G                                                                 | Ref. |
|--------------------|---------------|-------------|--------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|------|
| cell carcinoma     | serum         | 383 (controls) | ELISA       | Compared with the control group (6.45 ± 1.31 ng/L), the levels of sHLA-G in patients were significantly higher (8.25 ± 1.74 ng/L). | risk factors for the strong influence of tobacco on HNSCC in Indian. Potential diagnostic serum protein markers. | (129) |
|                    | (patients)    | 120         |              |                                                                                                 |                                                                                                |      |
|                    | (controls)    | 99          |              |                                                                                                 |                                                                                                |      |
| Esophageal cancer  | EDTA blood    | 230 (patients) | PCR         | In the Kazakh region, individuals with the -14 bp/-14 bp and C/C genotypes had a 2.82 times higher risk of developing EC than those with the +14 bp/+14 bp and C/C genotypes. | The 14 bp deletion/insertion of HLA-G gene may play a role in EC susceptibility of Kazakh.      | (130) |
|                    | (controls)    | 467         |              |                                                                                                 |                                                                                                |      |

and CA19-1, can improve the clinical screening of gastric cancer compared with sHLA-G alone (132). In patients with breast cancer, the expression of HLA-G is negatively correlated with proliferation factor (133). In addition, the concentration of sHLA-G in plasma helps predict and diagnose cancers of different subtypes. Compared with patients with simple lobular carcinoma and simple ductal carcinoma, patients with mixed lobular lesions and breast ducts have a more significant increase in sHLA-G (131). ELISA also shows that the plasma levels of sHLA-G in patients with liver cancer are higher compared with the control group (134). These results support that sHLA-G can be used for tumor early diagnosis.

In terms of prognosis, the high expression of sHLA-G in patients with non-small cell lung cancer is significantly related to poor overall survival (OS), especially in patients with advanced cancer (135). An interesting phenomenon has been found in the Tunisian population that patients with HLA-G*01:04:01 alleles have elevated plasma levels of sHLA-G, while patients who do not carry HLA G*0105N alleles (cannot encode HLA-G1 protein) express a dramatically reduced level of sHLA-G in plasma (22). In CRC, the prognostic value of plasma sHLA-G is mainly reflected by predicting the risk of liver metastasis in patients with stage II and III CRC. Hauben and colleagues have found that the sHLA-G levels are associated with shorter liver metastasis-free survival (LMFS) in patients with stage II CRC and longer LMFS in patients with stage III CRC. The possible reason can be attributed to the fact that the stage III patients receive chemotherapy before the sample collection, and the tumor cells are damaged (64). Therefore, the differential level of plasma sHLA-G is a predictive biomarker of treatment response to advanced CRC, and it is also a potential prognostic marker.

sHLA-G IN BODY FLUIDS OF TUMOR PATIENTS AS TUMOR MARKERS

The source of sHLA-G is related to the inflammatory factors present in the cancer microenvironment and the reduction of NK cells and memory T cells (136, 137). Researchers have revealed that the expression of HLA-G in ascites, saliva, and bronchial lavage fluid can be useful for tumor diagnosis and prognosis (Table 1).

ASCITES

Results obtained from Ullah’s group have shown that sHLA-G in ascites is mainly expressed by ascites cells, tumor cells, and stromal cells (39). Moreover, Sun et al. have found that the level of sHLA-G in malignant ascites induced by various solid tumors is significantly higher compared with benign ascites. Especially for ascites caused by gynecological tumors and gastrointestinal tumors, the levels of related tumor markers CEA and CA199 are also elevated in malignant ascites, while its specificity and sensitivity are significantly lower compared with sHLA-G (139). The level of sHLA-G in malignant ascites caused by OC and breast cancer is significantly higher compared with benign ascites. In addition, the specificity, sensitivity, and AUC are greatly improved when the critical value of 13 ng/mL is achieved. Besides, in OC, sHLA-G1/G5 is negatively correlated with CD3-/CD56+ subgroups and CD4+ CD45RO+ memory cells, suggesting that sHLA-G plays a role in the tumor microenvironment by up-regulating T-reg cells and down-regulating NK cells (39). In cytology-negative malignant ascites, sHLA-G also has outstanding diagnostic performance. In 32 cases of cytology-negative ascites, the positive rate of sHLA-G is 75%, which is significantly higher compared with CEA and CA199 (50). Collectively, sHLA-G can be used as an independent indicator for early diagnosis of malignant ascites, and it is helpful to screen for malignant ascites when cytology is negative.

SALIVA

Saliva is a body fluid that can be collected easily, quickly, and non-invasively (140). Biomolecules in saliva have been used as promising markers for early diagnosis, detection, and treatment in OSCC (141, 142), breast cancer (143, 144), OC (145), and other tumors. In OSCC, saliva is the body fluid that has the closest contact with oral tumors. Researchers have collected the saliva samples from 22 OSCC patients. Compared with non-metastatic OSCC, the HLA-G level of metastatic OSCC is dramatically elevated and correlated with poor OS (63). The sHLA-G level in CRC patients is also significantly higher compared with healthy controls, especially in patients with stage III-IV tumor (62). In the bronchial lavage fluid of
patients with different histological types of lung cancer, the level of sHLA-G is significantly correlated with lower Karnofsky index in metastatic tumors and can be used as a prognostic marker for lung cancer (24). The exudate of OC patients is collected before, during, or after chemotherapy. It is found that the expression of HLA-G is decreased after chemotherapy, and the decreased expression of HLA-G predicts the improvement of patient survival rate. This may be related to the preferential sensitivity of HLA-G-expressing cells (47). Therefore, researchers believe that saliva sHLA-G can be used as a diagnostic and prognostic biomarker for multiple cancers, and it is a less invasive alternative to venipuncture. However, more studies are needed to confirm the significance of saliva sHLA-G as a tumor indicator.

**HLA-G DERIVED FROM EVs AS A TUMOR MARKER**

EVs are composed of growth factors, biologically active lipids, genetic information, and antibodies/ligands/receptors, and they are resistant to RNase, enduring their great potential as a tumor biomarker (146–148). Secreted HLA-G can exist in the form of free sHLA-G or be secreted by EVs and found in various body fluids, such as plasma, ascites, and pleural exudate (96) (Figure 2). Recent studies have found that tumor cells, cytотrophoblast cells, and mesenchymal stem cells can secrete HLA-G-carrying EVs, playing a role in regulating the tumor microenvironment and immunosuppressive function (149). Therefore, HLA-G can be shedding from the cell surface by metalloproteases or released from various cells, incorporated into EVs, and serve as a promising tumor indicator (77, 104, 150).

Riteau et al. have isolated an HLA-G-positive cell line from primary and metastatic lymph node melanomas and named it Fon. For the first time, this melanoma cell line is found to secrete exosomes containing HLA-G1. They speculate that the immune tolerance produced by melanoma-derived HLA-G exosomes may be a method for tumors to regulate host immunity. So far, the specific mechanism of this method is still unclear (150). In breast cancer patients receiving neoadjuvant chemotherapy (NACT), the relationship between exosomes carrying HLA-G and the prognosis of the disease has been evaluated. Before NACT, the sHLA-G<sub>EV</sub> levels are correlated with circulating stem cell-like tumor cells. The total amount of sHLA-G<sub>EV</sub> is

### TABLE 2 | Clinical research involving diagnosis and prognosis of HLA-G in the body fluid.

| Cancer type     | Sample source     | Sample size | HLA-G type | Methods (Ab)       | Experimental results evaluation                              | Expression evaluation of HLA-G | Ref. |
|-----------------|-------------------|-------------|------------|--------------------|----------------------------------------------------------------|-------------------------------|------|
| Breast cancer   | ascites           | 24          | sHLA-G     | ELISA (W6/32)      | The levels of sHLA-G were significantly higher in malignant compared with benign ascites | Measurement of sHLA-G is a useful molecular adjunct to cytology in the differential diagnosis of malignant versus benign ascites | (50) |
| Ovarian cancer  | ascites           | 19          | sHLA-G     | ELISA (MEM-G/9, 5A6G7) | The level of HLA-G5 isoform was specifically higher in seven samples. | sHLA-G, particularly HLA-G5, may affect antitumor immune response both in situ and in circulation. | (39) |
| Ovarian cancer  | peritoneal fluid  | 16          | sHLA-G     | IHC (4H84)         | IHC showed predominantly focal HLA-G expression in 12 of 46 (26%) breast carcinoma effusions and 16 of 39 (41%) solid lesions | Associated with shorter disease-free survival. | (51) |
| Breast cancer   | effusions         | 46          | HLA-G      | IHC (4H84)         | HLA-G was detected in cancer cells in 49/148 (33%) effusions, 33/66 (50%) primary tumors, and 59/122 (48%) solid metastases. | A new role for HLA-G as a prognostic indicator in advanced-stage OC in effusions. | (47) |
| Ovarian cancer  | effusions         | 148         | HLA-G      | IHC (4H84)         | HLA-G was detected in cancer cells in 49/148 (33%) effusions, 33/66 (50%) primary tumors, and 59/122 (48%) solid metastases. | sHLA-G can be a good prognostic and diagnostic biomarker in CRC. | (62) |
| Colorectal cancer | saliva      | 20(patients) | sHLA-G     | ELISA             | In patients diagnosed with CRC, salivary sHLA-G values were significantly higher compared with the control group of healthy patients | sHLA-G is closely associated with the survival of CRC patients. | (135) |
| Colorectal cancer | single-cell suspensions | 157 | HLA-G | Flow Cytometry Analysis (MEM-G/9) | The median percentage of HLA-G expression was 14.90% (range:1.81% to 79.90%). | sHLA-G can be a good prognostic and diagnostic biomarker in CRC. | (62) |
| Oral squamous cell cancer | saliva | 22(patients) | sHLA-G | ELISA | There was no significant difference in sHLA-G concentration between OSCC and control groups. | It helps tumor cells evade immune defense mechanisms. | (63) |
| Lung cancer     | bronchoalveolar fluid | 31 | sHLA-G     | ELISA            | The mean value of soluble HLA-G was 49.04 ng/mL, and the level of HLA G varied greatly in metastatic tumors. | HLA-G soluble protein is significantly associated with patients with metastatic tumor and can be used as a prognostic marker of lung cancer. | (24) |
significantly increased after NACT, and it is related to the disease process, while the total sHLA-G level has nothing to do with the clinical prognosis (48). In addition, HLA-G-bearing EVs released by renal cancer cells damage the differentiation of monocytes into DCs and inhibit the maturation process of DCs. These findings suggest that HLA-G-mediated tumor immune escape mechanisms can spread to HLA-G-negative tumor cells through the EV pathway (151, 152). In epithelial ovarian cancer (EOC), the levels of HLA-G<sub>EV</sub> are increased by seven times compared with healthy controls, and the elevated HLA-G<sub>EV</sub> can serve as independent 3-year and 10-year progression-free survival (PFS) prognostic factors. It is worth noting that all patients with high levels of HLA-G<sub>EV</sub> experience disease progression within approximately 5 years after the initial diagnosis. Schwich's group has also shown that HLA-G<sub>EV</sub> serves as an independent risk assessment marker for disease progression of EOC (40).

**HLA-G DETECTION AND QUANTIFICATION**

Based on the current understanding of HLA-G and its various forms that have multiple immune tolerance regulating functions in malignant tumors, HLA-G is generally recognized as a biomarker that can be used to monitor the disease state and progression in cancer patients (153). However, due to the diversity of HLA-G structures, the standardization of HLA-G detection methods has been a topic of discussion from the past to the present (154). HLA-G test results vary greatly in different locations of HLA-G acquisition, between different tumors, or the same tumor in different laboratory test results.

**DETECTION OF HLA-G IN TISSUES**

The status of HLA-G in tumor tissues is usually detected by IHC. Although IHC is an experimental technique that has been widely used, there is controversy about the use of such a method to detect HLA-G. Because HLA-G has multiple subtypes, and each antibody recognizes only specific epitopes, leading to different staining results (52, 54, 124, 155). In addition, the experimental procedures of IHC also differ greatly, such as the type of antibody used and its dilution, incubation time, and staining evaluation criteria. The differences in treatment history, tumor subtypes, and individual tumor microenvironment between different patients will also affect the evaluation of HLA-G expression levels (156–158).

In 81 patients with colon cancer, Swets and colleagues have used three monoclonal antibodies (4H84, MEM-G/1, and MEM-G/2) to evaluate the expression of HLA-G. In primary tumors, the positive staining rates of HLA-G using monoclonal antibody 4H84, MEM-G/1, and MEM-G/2 are 29%, 6%, and 10%, respectively. They have found that different epitopes of HLA-G detected by different monoclonal antibodies are differentially expressed in CRC tissues (159–161). Although it has been confirmed that 4H84 and MEM-G/1 can recognize all subtypes of HLA-G, the reason for this difference in expression is generally believed to be a cross-reaction with HLA-I (160, 161). For example, 4H84 has been shown to cross-react with the existence of β2M free classic HLA class I molecules on activated leukocytes. This may lead to an overestimation of HLA-G expression in pathological tissues recognized by leukocyte infiltration (such as CRC), leading to differences among studies (162). Therefore, it is recommended to use a variety of different monoclonal antibodies to detect HLA-G.

In addition, whether these antibodies can block the binding of HLA-G to its receptors (ILT2, ILT4, and KIR2DL4) is also a challenge (163). Antibodies targeting the α3 domain and β2M of HLA-G can block the binding of HLA-G to ILT2 and ILT4. The antibody targets the α2 domain and can block the binding of HLA-G to KIR2DL4. It has been confirmed that MEM-G/1 blocks the binding site of HLA-G2 and ILT4, while MEM-G/9 and G233 can bind to HLA-G1, depriving the binding site of ILT2. Besides, antibody 87G can block the interaction between HLA-G1 and its receptor (164). Therefore, to block the interaction of HLA-G with all its receptors, it is necessary to develop an antibody mixture that can recognize all HLA-G subtypes and block the binding of HLA-G to ILT2, ILT4, and KIR2DL4, thereby reducing HLA-G cross-reaction with its receptors.

**DETECTION OF sHLA-G IN FLUIDS**

sHLA-G is also expressed and released by cancer cells, which is a potential biomarker in the body fluids of cancer patients (62). In particular, sHLA-G in the supernatant of IVF embryos has been considered as an independent factor predicting pregnancy outcome (165–167). Therefore, as early as 2004, in the Wet Workshop for the quantification of sHLA-G, the standardization of sHLA-G detection and quantification methods has been discussed, including sensitivity, standard reagents, and antibody specificity (168). At present, ELISA is mainly employed to quantify the level of sHLA-G in body fluids. The monoclonal antibody MEM-G/9 is used to simultaneously detect the shedding of HLA-G1 and the secreted HLA-G5. MEM-G/9 specifically captures β2M-related HLA-G1 and HLA-G5 as well as polyclonal anti-human β2M antibodies (169). In this seminar, HLA-G-expressing EVs are also discovered. This structure cannot be detected by the combination of MEM-G/9 and anti-β2 but can be detected by an antibody combination of mAb 5A6G7 and W6/32. Given the often-antagonistic composition and complex structure of EVs, the various components carried by EVs may drive the functional activity of EVs expressing HLA-G and eliminate or enhance the immune tolerance function of HLA-G.

ExoQuick™ precipitation is now usually used to extract EVs from samples and to quantify the number of EV particles and the number of vesicular-bound HLA-G (HLA-G<sub>EV</sub>) (48). The results show that in OC, poor clinical status and presence of CTC, and PFS are associated with elevated HLA-G<sub>EV</sub> levels (40). In breast cancer patients receiving NACT, high levels of vesicular sHLA-G are also associated with disease progression (48). Given these,
future work should focus on the standardization process. Before being applied to routine clinical practice, a larger research cohort, prospective research, and internationally recommended standardized testing methods are needed to verify the application of HLA-G in diseases. Importantly, it is necessary to develop more specific antibodies against HLA-G subtypes, explore new undiscovered HLA-G subtypes, and make full use of existing experimental techniques to evaluate the role of various subtypes in various tumors.

CONCLUSIONS
After more than 30 years of research, it has been shown that HLA-G has unique characteristics as follows: 1) it participates in the immune tolerance network in healthy individuals and tumor patients; 2) it is barely expressed in normal tissues and frequently identified in tumor cells. Given these characteristics of HLA-G, one of the current research goals is to serve as a biomarker for diagnosis, prognosis, and clinical testing. However, many factors affect the interpretation of the clinical significance of HLA-G, such as the number of patients included in the study, different clinical parameters of patients (such as medical history, disease stage, and treatment plan), and differences in testing protocols. To better explain the structural diversity of HLA-G, sHLA-G, HLA-GIV, and their expression and clinical significance in tumors, future efforts should be devoted to studies focusing on multicenter and large sample analysis, and the establishment of standardized and feasible HLA-G detection methods.

AUTHOR CONTRIBUTIONS

PL, NW and YZ wrote and edited the manuscript. CW and LD edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING
This research was supported by grant from the National Natural Science Foundation of China (82002228 to PL, 82072368 to LD), the Key Research and Development Program of Shandong Province (2019GSF108091 to LD), Taishan Scholars Program of Shandong Province awarded to CXW and LD.

REFERENCES

1. Ellis SA, Sargent IL, Redman CW, McMichael AJ. Evidence for a Novel HLA Antigen Found on Human Extravillous Trophoblast and a Choriocarcinoma Cell Line. Immunology (1986) 59(4):595–601.
2. Heinrichs H, Orr HT. HLA Non-A,B,C Class I Genes: Their Structure and Expression. Immunol Rev (1990) 9(4):263–74. doi: 10.1007/BF02935526
3. Geraghty DE, Koller BH, Orr HT. A Human Major Histocompatibility Complex Class I Gene That Encodes a Protein With a Shortened Cytoplasmic Segment. Proc Natl Acad Sci USA (1987) 84(24):9145–9. doi: 10.1073/pnas.84.24.9145
4. Shiroishi M, Kuroki K, Ose T, Rasubala L, Shiratori I, Arase H, et al. Characteristics of HLA-G in Diseases. Importantly, it is necessary to develop more specific antibodies against HLA-G subtypes, explore new undiscovered HLA-G subtypes, and make full use of existing experimental techniques to evaluate the role of various subtypes in various tumors.

CONCLUSIONS
After more than 30 years of research, it has been shown that compared with other tumor markers, HLA-G has unique characteristics as follows: 1) it participates in the immune tolerance network in healthy individuals and tumor patients; 2) it is barely expressed in normal tissues and frequently identified in tumor cells. Given these characteristics of HLA-G, one of the current research goals is to serve as a biomarker for diagnosis, prognosis, and clinical testing. However, many factors affect the interpretation of the clinical significance of HLA-G, such as the number of patients included in the study, different clinical parameters of patients (such as medical history, disease stage, and treatment plan), and differences in testing protocols. To better explain the structural diversity of HLA-G, sHLA-G, HLA-GIV, and their expression and clinical significance in tumors, future efforts should be devoted to studies focusing on multicenter and large sample analysis, and the establishment of standardized and feasible HLA-G detection methods.

AUTHOR CONTRIBUTIONS

PL, NW and YZ wrote and edited the manuscript. CW and LD edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING
This research was supported by grant from the National Natural Science Foundation of China (82002228 to PL, 82072368 to LD), the Key Research and Development Program of Shandong Province (2019GSF108091 to LD), Taishan Scholars Program of Shandong Province awarded to CXW and LD.

REFERENCES

1. Ellis SA, Sargent IL, Redman CW, McMichael AJ. Evidence for a Novel HLA Antigen Found on Human Extravillous Trophoblast and a Choriocarcinoma Cell Line. Immunology (1986) 59(4):595–601.
2. Heinrichs H, Orr HT. HLA Non-A,B,C Class I Genes: Their Structure and Expression. Immunol Rev (1990) 9(4):263–74. doi: 10.1007/BF02935526
3. Geraghty DE, Koller BH, Orr HT. A Human Major Histocompatibility Complex Class I Gene That Encodes a Protein With a Shortened Cytoplasmic Segment. Proc Natl Acad Sci USA (1987) 84(24):9145–9. doi: 10.1073/pnas.84.24.9145
4. Shiroishi M, Kuroki K, Ose T, Rasubala L, Shiratori I, Arase H, et al. Characteristics of HLA-G in Diseases. Importantly, it is necessary to develop more specific antibodies against HLA-G subtypes, explore new undiscovered HLA-G subtypes, and make full use of existing experimental techniques to evaluate the role of various subtypes in various tumors.

CONCLUSIONS
After more than 30 years of research, it has been shown that compared with other tumor markers, HLA-G has unique characteristics as follows: 1) it participates in the immune tolerance network in healthy individuals and tumor patients; 2) it is barely expressed in normal tissues and frequently identified in tumor cells. Given these characteristics of HLA-G, one of the current research goals is to serve as a biomarker for diagnosis, prognosis, and clinical testing. However, many factors affect the interpretation of the clinical significance of HLA-G, such as the number of patients included in the study, different clinical parameters of patients (such as medical history, disease stage, and treatment plan), and differences in testing protocols. To better explain the structural diversity of HLA-G, sHLA-G, HLA-GIV, and their expression and clinical significance in tumors, future efforts should be devoted to studies focusing on multicenter and large sample analysis, and the establishment of standardized and feasible HLA-G detection methods.

AUTHOR CONTRIBUTIONS

PL, NW and YZ wrote and edited the manuscript. CW and LD edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING
This research was supported by grant from the National Natural Science Foundation of China (82002228 to PL, 82072368 to LD), the Key Research and Development Program of Shandong Province (2019GSF108091 to LD), Taishan Scholars Program of Shandong Province awarded to CXW and LD.
55. Tawfeek GA, Alhassanin S. HLA-G Gene Polymorphism in Egyptian Patients With Non-Hodgkin Lymphoma and Its Clinical Outcome. *Immunol Invest* (2018) 47(3):315–25. doi: 10.1080/08822339.2018.1430826

56. Gros F, Sehti V, Guibert B, Branger B, Bernard M, Fauchet R, et al. Soluble HLA-G Molecules Increase During Acute Leukemia, Especially in Subtypes Affecting Monocytic and Lymphoid Lineages. *Neoplasia* (New York NY) (2006) 8(3):223–30. doi: 10.1593/neo.050703

57. Urosevic M, Kamarasev J, Burg G, Dummer R. Primary Cutaneous CD8+ and CD56+ T-Cell Lymphomas Express HLA-G and Killer-Cell Inhibitory Ligand, Il2c. *Blood* (2004) 103(5):1796–8. doi: 10.1182/blood-2003-10-3372

58. Chen J, Shen B, Jiang Y, Jun L, Zhu M, Chen B, et al. Analysis of Immunoglobulin-Like Transcripts (ILTs) in Lymphocytes With HLA-G and IL10 From SLE Patients. *Clin Exp Med* (2013) 13(2):135–42. doi: 10.1007/s10238-012-0185-6

59. Negrini S, Fenoglio D, Parodi A, Kalli F, Battaglia F, Nasi G, et al. Phenotypic Alterations Involved in CD8+ Treg Impairment in Systemic Sclerosis. *Front Immunol* (2017) 8:18. doi: 10.3389/fimmu.2017.00018

60. Lin A, Yan WH. Human Leukocyte Antigen-G (HLA-G) Expression in Colorectal Cancer Disease Stages. *J Hum Immunol* (2014) 81(4):127–33. doi: 10.1089/jhum.2014.02244

61. Jacobsen DP, Lekva T, Moe K, Fjeldstad HES, Johnsen GM, Suguille M, et al. Pregnancy and Postpartum Levels of Circulating Maternal sHLA-G in Preeclampsia. *J Reprod Immunol* (2021) 143:103249. doi: 10.1016/j.jri.2020.103249

62. Lázaro-Sánchez AD, Salces-Ortiz E, Velásquez LI, Orozco-Beltrán D, Díaz-Fernández N, Juarez-Marroqui A. HLA-G as a New Tumor Biomarker: Detection of Soluble Isoforms of HLA-G in the Serum and Saliva of Patients With Colorectal Cancer. *Clin Trans Oncol Off Publ Fed Spanish Oncol Societies Natl Cancer Institute Mexico* (2020) 22(7):1166–71. doi: 10.1007/s12094-019-02244-2

63. González AS, Arantes DA, Bernardes VF, Jaeger F, Silva JM, Silva TA, et al. Immunosuppressive Mediators of Oral Squamous Cell Carcinoma in Tumour Samples and Saliva. *Hum Immunol* (2015) 76(1):52–8. doi: 10.1016/j.humimm.2014.11.002

64. Kirana C, Ruszkiewicz A, Stubbs RS, Hardingham JE, Hewett PJ, Maddern GJ, et al. Soluble HLA-G A Is a Differential Prognostic Marker in Sequential Colorectal Cancer Disease Stages. *Int J Cancer* (2017) 140(11):2577–86. doi: 10.1002/ijc.30667

65. Bertol BC, de Araújo JNG, Sadissou IA, Sonon P, Dias FC, Bortolin RH, et al. Plasma Levels of Soluble HLA-G and Cytokines in Papillary Thyroid Carcinoma Before and After Thyroidectomy. *Int J Clin Pract* (2020) 74(10):e15385. doi: 10.1111/ijs.15385

66. Sahay M, Boujelbene N, Ben Yahia H, Bertolotti D, Zenni I, Ouzari HI, et al. Prognostic Significance of High Circulating sHLA-G in Ovarian Carcinoma. *Hla* (2021) 98(4):357–65. doi: 10.1111/tan.14374

67. Dardano A, Rizzo R, Polini A, Stignani M, Tognini S, Pasqualetti G, et al. Soluble Human Leukocyte Antigen-G and Its Insertion/Deletion Polymorphism in Papillary Thyroid Carcinoma: Novel Potential Biomarkers of Disease? *J Clin Endocrinol Metab* (2012) 97(11):4080–6. doi: 10.1210/jc.2012-2231

68. Zheng J, Xu C, Chu D, Zhang X, Li J, Ji G, et al. Human Leukocyte Antigen G Is Associated With Esophageal Squamous Cell Carcinoma Progression and Poor Prognosis. *Immunol Lett* (2014) 161(1):13–9. doi: 10.1016/j.imlet.2014.04.007

69. Xu YF, Lu Y, Cheng H, Jiang J, Xu J, Long J, et al. High Expression of Human Leukocyte Antigen-G Is Associated With a Poor Prognosis in Patients With PDAC. *Carr Mol Med* (2015) 15(4):360–7. doi: 10.2174/15656201566615041102218

70. Mallet V, Blaschitz V, Crisa L, Schmitt C, Fournel S, King A, et al. HLA-G in the Human Thymus: A Subpopulation of Medullary Epithelial But Not CD83(+) Dendritic Cells Expresses HLA-G as a Membrane-Bound and Soluble Protein. *Int Immunol* (1999) 11(6):889–98. doi: 10.1093/immunim/11.6.889

71. Larsen MH, Bzorek M, Pass MB, Larsen LG, Nielsen MW, Svedensg SG, et al. Human Leukocyte Antigen-G in the Male Reproductive System and in Seminal Plasma. *Mol Hum Reprod* (2011) 17(12):727–38. doi: 10.1093/molehr/gar052
90. Lee CL, Guo Y, So KH, Vijayan M, Guo Y, Wong VH, et al. Soluble Human Leukocyte Antigen G5 Polyzymes Differentiation of Macrophages Toward a Decisional Macrophage-Like Phenotype. *Hum Reprod (Oxford England)* (2012) 27(10):2263–74. doi: 10.1093/humrep/der196

91. Morandi F, Rouas-Freiss N, Pistoia V. The Emerging Role of Soluble HLA-G in the Control of Chemotaxis. *Cytokine Growth Factor Rev* (2014) 25(3):327–35. doi: 10.1016/j.cytogfr.2014.04.004

92. Gregori S, Tomsan M, Paccioli V, Scirpoli M, Battaglia M, Magnani CF, et al. Differentiation of Type 1 T Regulatory Cells (T1) by Tolerogenic DC-10 Requires the IL-10-Dependent ILT4/HLA-G Pathway. *Blood* (2010) 116(6):935–44. doi: 10.1182/blood-2009-07-234872

93. Fainardi E, Castellazzi M, Stignani M, Morandi F, Sana G, Gonzalez R, et al. Emerging Topics and New Perspectives on HLA-G. *Cell Mol Life Sci CMLS* (2011) 68(5):433–51. doi: 10.1007/s00018-010-0584-3

94. Carosella ED, Rouas-Freiss N, Tronik-Le Roux D, Moreau P, LeMaoult J. HLA-G: An Immune Checkpoint Molecule. *Adv Immunol* (2015) 127:33–144. doi: 10.1016/bs.ai.2015.04.001

95. Lin A, Yan WH. Intercellular Transfer of HLA-G: Its Potential in Cancer Immunology. *Clin Trans Immunol* (2019) 8(9):e1077. doi: 10.1016/j.citi.2017.05.008

96. Alegre E, Rebmann V, Lemaoult J, Rodriguez C, Horn PA, Diaz-Lagares A, et al. *In Vivo Identification of an HLA-G Complex as Ubiquitinated Protein Circulating in Eosinomes*. *Euro J Immunol* (2015) 45(7):1933–9. doi: 10.1002/eji.201343318

97. T1F1 Ta K, Kabani J, Favaloro J, Yang S, Ho PJ, Gibson J, et al. CD86+ or HLA-G+ can be Transferred via Trogocytosis From Myeloma Cells to T Cells and Are Associated With Poor Prognosis. *Blood* (2012) 120(10):2055–63. doi: 1182/blood-2012-03-341792

98. LeMaoult J, Caumartin J, Daouy M, Switala M, Rebmann V, Arnulf B, et al. Trogocytic Intercellular Membrane Exchanges Among Hematological Tumors. *J Hematol Oncol* (2015) 8:24. doi: 10.1186/s13045-015-0114-8

99. Tilburgs T, Evans JH, Crespo A, Strominger JL. The HLA-G Cycle: Provides for Both NK Tolerance and Immunity at the Maternal-Fetal Interface. *Proc Natl Acad Sci USA* (2015) 112(33):13312–7. doi: 10.1073/pnas.1517724112

100. Han L, Lam EW, Sun Y. Extracellular Vesicles in the Tumor Microenvironment: Old Stories, But New Tales. *Mol Cancer* (2019) 18(1):59. doi: 10.1186/s12934-019-0980-8

101. Simon T, Jackson E, Giamas G. Breaking Through the Gliallostroma Microenviroment via Extracellular Vesicles. *Oncogene* (2020) 39(23):4477–90. doi: 10.1080/13540538.2020.1308-2

102. Colombo M, Raposo G, Thery C. Biogenesis, Secretion, and Intercellular Interactions of Exosomes and Other Extracellular Vesicles. *Annu Rev Cell Dev Biol* (2014) 30:255–89. doi: 10.1146/annurev-cellbio-101512-122326

103. Wu T, Dai Y. Tumor Microenvironment and Therapeutic Response. *Cancer Lett* (2017) 387:61–8. doi: 10.1016/j.canlet.2016.01.043

104. Curigliano G, Crucitti C, Gela L, Goldhirsch A. Molecular Pathways: Human Leukocyte Antigen G (HLA-G). *Clin Cancer Res* (2013) 19(20):5564–71. doi: 10.1158/1058-8278.CCR-12-3697

105. Amiot L, Vu N, Samson M. Biology of the Immunomodulatory Molecule Human Leukocyte Antigen G5 Polarizes Differentiation of Macrophages Toward a Decisional Macrophage-Like Phenotype. *Hum Reprod* (2015) 30:255–6. doi: 10.1038/humrep.de2016.183

106. Brown R, Kabani K, Favaloro J, Yang S, Ho PJ, Gibson J, et al. CD86+ or HLA-G+ can be Transferred via Trogocytosis From Myeloma Cells to T Cells and Are Associated With Poor Prognosis. *Blood* (2012) 120(10):2055–63. doi: 10.1182/blood-2012-03-341792

107. LeMaoult J, Caumartin J, Daouy M, Switala M, Rebmann V, Arnulf B, et al. Trogocytic Intercellular Membrane Exchanges Among Hematological Tumors. *J Hematol Oncol* (2015) 8:24. doi: 10.1186/s13045-015-0114-8

108. Tilburgs T, Evans JH, Crespo A, Strominger JL. The HLA-G Cycle: Provides for Both NK Tolerance and Immunity at the Maternal-Fetal Interface. *Proc Natl Acad Sci USA* (2015) 112(33):13312–7. doi: 10.1073/pnas.1517724112

109. Han L, Lam EW, Sun Y. Extracellular Vesicles in the Tumor Microenvironment: Old Stories, But New Tales. *Mol Cancer* (2019) 18(1):59. doi: 10.1186/s12934-019-0980-8

110. Simon T, Jackson E, Giamas G. Breaking Through the Gliallostroma Microenviroment via Extracellular Vesicles. *Oncogene* (2020) 39(23):4477–90. doi: 10.1080/13540538.2020.1308-2

111. Khodabandeh Shahraki P, Zare Y, Azarpira N, Hosseinzadeh M, Farjadian S. Correlation Between HLA-G Polymorphism, Tobacco Consumption and Risk of Head and Neck Squamous Cell Carcinoma (HNSCC) in North Indian
Cai MY, Xu YF, Qiu SJ, Ju MJ, Gao Q, Li YW, et al. Human Leukocyte Antigen-G Expression in Tumor Tissues and Soluble HLA-G Plasma Levels in Patients With Gastrointestinal Cancer. Asian Pacific J Cancer Prev APJCP (2018) 19(10):2731–5. doi: 10.22034/APJCP.2018.19.10.2731

Wuerfel FM, Hueber H, Häberle L, Gessi M, Alegre E, et al. The Immunogenicity of HLA-G and Its Receptors: Where to Intervene for Cancer Immunotherapy? Curr Immunol Rep (2020) 21(22):8678. doi: 10.3390/cir.21.22.8678
164. Furukawa A, Meguro M, Yamazaki R, Watanabe H, Takahashi A, Kuroki K, et al. Evaluation of the Reactivity and Receptor Competition of HLA-G Isoforms Toward Available Antibodies: Implications of Structural Characteristics of HLA-G Isoforms. *Int J Mol Sci* (2019) 20(23):5947. doi: 10.3390/ijms20235947

165. Rebmann V, Switala M, Eue I, Grosse-Wilde H. Soluble HLA-G Is an Independent Factor for the Prediction of Pregnancy Outcome After ART: A German Multi-Centre Study. *Hum Reprod (Oxford England)* (2010) 25(7):1691–8. doi: 10.1093/humrep/deq120

166. Rebmann V, Switala M, Eue I, Schwahn E, Merzenich M, Grosse-Wilde H. Rapid Evaluation of Soluble HLA-G Levels in Supernatants of In Vitro Fertilized Embryos. *Hum Immunol* (2007) 68(4):251–8. doi: 10.1016/j.humimm.2006.11.003

167. Rizzo R, Vercammen M, van de Velde H, Horn PA, Rebmann V. The Importance of HLA-G Expression in Embryos, Trophoblast Cells, and Embryonic Stem Cells. *Cell Mol Life Sci CMLS* (2011) 68(3):341–52. doi: 10.1007/s00018-010-0578-1

168. Rebmann V, LeMaoult J, Rouas-Freiss N, Carosella ED, Grosse-Wilde H. Report of the Wet Workshop for Quantification of Soluble HLA-G in Essen, 2004. *Hum Immunol* (2005) 66(8):853–63. doi: 10.1016/j.humimm.2005.05.003

169. Rebmann V, LeMaoult J, Rouas-Freiss N, Carosella ED, Grosse-Wilde H. Quantification and Identification of Soluble HLA-G Isoforms. *Tissue Antigens* (2007) 69 Suppl 1:143–9. doi: 10.1111/j.1399-0039.2006.763_5.x

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Li, Wang, Zhang, Wang and Du. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.