Review

Human Leukocyte Antigen-G (HLA-G)
as a marker for diagnosis, prognosis and
tumor immune escape in human malignancies

Shang-mian Yie[1] and Zhenbo Hu[2]

[1]Research Center, The Second Medical College/Teaching Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan, PR. China and [2]Translational Hematology and Oncology Research, Taussig Cancer Center, Cleveland Clinic, Cleveland, Ohio, USA

Summary. Human leukocyte antigen-G (HLA-G) is a non-classical HLA-class Ib molecule with multiple immunoregulatory properties. Its main functions in physiological conditions are to abolish maternal immune cell activity against fetus and to establish immune tolerance at the maternal-fetal interface. In oncology, HLA-G molecules are aberrantly expressed in a variety of human neoplastic diseases and play an important role in the escape of tumor cells from immune surveillance. In the past few years, making use of HLA-G protein expression in tissues and circulating levels in body fluids as a tumor marker have been the focus of extensive research in the diagnosis and prognosis of several human malignancies. In addition, this molecule might be a promising target for future immune therapeutic approaches based on its immune tolerant functions and its highly specific expression for malignant transformation. In this review, we will summarize available literature data as well as our own works on HLA-G in cancer, and address some of the issues concerning its application in human neoplasia.

Key words: HLA-G, Tumor marker, Diagnosis, Prognosis, Tumor immune escape, Immunotherapy

Offprint requests to: Shang-mian Yie, Ph.D., Research Center, The Second Medical College/Teaching Hospital, Chengdu University of Traditional Chinese Medicine, No15, The Fourth Segment, Remin Nanlu Road , Chengdu, Sichuan, PR. China. e-mail: shangmian.yie@gmail.com

Introduction

Cancer is essentially considered a complex cell disease caused by abnormalities in the genetic material of transformed cells. However, cancer development is a complicated progressive process that involves a sequence of gene-environment interactions with dysfunctions in multiple systems, including immune functions. The immune system can specifically identify and eliminate tumor cells based on their expression of tumor-specific antigens or molecules induced during malignant cell transformation. This process is referred to as tumor immune surveillance (Swann and Smyth, 2007). Despite tumor immune surveillance, tumors can still develop in the presence of a functioning immune system. Therefore, cancer can be explained, at least in part, as an abnormal immune system tolerance to uncontrolled cells (de la Cruz-Merino et al., 2008).

To explain the role of the immune system in tumor development, an updated concept of tumor immunoediting was introduced (Dunn et al., 2002). This concept consists of three phases: elimination, equilibrium, and escape (Dunn et al., 2002, 2004). In the elimination phase, the immune system detects and eliminates tumor cells that have developed because of failed intrinsic tumor suppressor mechanisms. The elimination phase is considered complete when all tumor cells are eliminated, or incomplete when only a portion of the tumor cells is eliminated. In the case of partial tumor elimination, the theory of immunoediting suggests that a temporary state of equilibrium can develop between the immune system and the developing tumor. In this state, it is predicted that tumor cells either remain dormant or continue to evolve by accumulating further

changes, such as DNA mutations or changes in gene expression, although the immune system still has sufficient control over tumor progression. Eventually, however, if the immune response fails to eliminate the tumor the result is the development of tumor cell variants that are able to resist, avoid, or even suppress the anti-tumor immune response, thereby leading to the escape phase. During this phase, the immune system is no longer able to contain the progressive growth of the tumor.

Several mechanisms for tumor escape have been proposed both acting locally - in the tumor microenvironment - and systemically (Laheru and Jafee, 2007). One of these concerns the expression of immune-modulatory molecules in the tumor microenvironment and soluble suppressive factors by tumor cells (Swann and Smyth, 2007). HLA-G is one such immune-modulatory molecule that is involved in every phase of cancer immunoediting (Urosevic and Dummer, 2008).

HLA-G is a non-classical HLA class I molecule that differs from classical HLA class I molecules by its restricted distribution to immune privileged sites such as trophoblast (Menier et al., 2003), thymus (Crisa et al., 1997), cornea (Le Discorde et al., 2003), pancreas (Cirulli et al., 2005) and mesenchymal stem cells (Selmani et al., 2008, 2009). HLA-G plays an important role in immunotolerance mechanisms by inhibiting the activity of tumor-infiltrating NK cells, cytotoxic T lymphocytes and APC through binding with inhibitory receptors present in immune cells such as ILT-2 (LILRB1/CD85j), ILT-4 (LILRB2/CD85d) and KIR2DL4 (CD158d) (Carosella et al., 2008a). The immunoinhibitory properties of HLA-G are believed to be beneficial in pregnancy, organ transplantation and autoimmune disease by promoting uterine implantation of the embryo, accepting solid allografts, and turning down immune reaction against self-components. However, they become deleterious in cancer and viral infections by allowing tumor cells and viruses to evade anti-tumor or anti-viral responses (Carosella et al., 2008a). Consequently, these biological functions of HLA-G may play an important role in clinical applications at the diagnostic, prognostic and therapeutic levels.

Concerning oncology, HLA-G expression has been detected in a variety of human neoplastic diseases *in situ*, but not in the surrounding normal tissues (Carosella et al., 2008a). In addition, soluble HLA-G (sHLA-G) is increased in body fluids when compared to normal healthy controls or benign disease cases (Pistoia et al., 2007). In the past few years, increasing evidence has shown that HLA-G can be used as a tumor immune escape-associated tumor marker for cancer diagnosis and prognosis, as well as a potential target for cancer immunotherapy. In this review, we summarize available literature data as well as our own works on HLA-G in cancer, and address some of the issues concerning its application in human neoplasia.

HLA-G as a prognostic marker for cancer patients

Ever since the first description of HLA-G expression in tumor cells (Paul et al., 1998), there have been numerous studies demonstrating that HLA-G gene transcription and protein are aberrantly expressed in various types of tumor lesions (Carosella et al., 2008a,b). Interestingly, the detection of HLA-G was reported to be correlated with certain clinicopathological parameters which are associated with disease progression in melanoma (Ugurel et al., 2001), ovarian and breast cancers (Singer et al., 2003; Davidson et al., 2005), lung cancer (Urosevic et al., 2001) and endometrial cancer (Barrier et al., 2006). These studies have indicated that HLA-G might serve as a clinical marker in the prediction of clinical outcomes for those diseases.

Indeed, as summarized in Table 1, abundant studies have established a correlation between HLA-G expression and patients' clinical outcome, including overall survival rate and the risk of developing metastatic disease. By using immunohistochemical (IHC) assay, Yie and colleagues demonstrated that HLA-G expression in tumors was significantly correlated with the clinical outcome of patients with colorectal cancer (CRC) (Ye et al., 2007), gastric cancer (GC) (Yie et al., 2007a), non-small cell lung cancer (NSCLC) (Yie et al., 2007b), esophageal squamous cell cancer (ESCC) (Yie et al., 2007c) and breast cancer (He et al., 2010). These diseases constitute some of the most common malignant cancers in the world and represent the most common causes of cancer-related deaths. Those studies also showed that the detection of HLA-G expression using IHC had a strong and independent prognostic value for the diseases.

Recently, Cai and colleagues observed that HLA-G expression detected by IHC was associated with the prognosis of hepatocellular carcinoma (HCC), especially in early-stage diseases with high expression levels independently associated with a shortened overall survival rate and increased tumor recurrence (Cai et al., 2009). The results were confirmed by another study performed by Lin and colleagues, in which HLA-G expression in HCC was strongly correlated with advanced disease stages (Lin et al., 2010a). This group also established that HLA-G expression in NSCLC lesions was strongly correlated with disease stages, and that patients with higher plasma sHLA-G levels had a significantly shorter survival time (Lin et al., 2010b).

Concerning malignant hematopoietic diseases, Nuckel and colleagues retrospectively investigated HLA-G expression in circulating B chronic lymphocytic leukemia (B-CLL) cells from 47 patients by the use of flow cytometry, and found that HLA-G-negative patients had a significantly longer progression-free survival time than HLA-G positive patients. In a multivariate analysis, HLA-G expression was shown to be an independent prognostic factor (Nuckel et al., 2005). Likewise, Erikci and colleagues also detected a statistically significant

correlation between positive HLA-G expression and progression-free survival in B-CLL patients (Erikci et al., 2009). Moreover, an elevated expression level of both membrane-bound and soluble HLA-G (sHLA-G) has been observed in multi-lineage hematopoietic malignancies. sHLA-G molecules seem more frequently expressed than membrane-bound isoforms during hematologic malignancies. The increased secretion of sHLA-G is more marked in acute leukemia subtypes, including monocytic and lymphoid lineages, as well as both B and T acute lymphoblastic leukemia (Gros et al., 2006). Sebti et al. (2007) studied sHLA-G expression in lymphoproliferative disorders and found that sHLA-G plasma level was significantly increased in 110 of 178 newly diagnosed lymphoid proliferation cases, including chronic lymphocytic leukaemia (CLL), B non-Hodgkin lymphomas (NHL) and T-NHL.

Most published data have demonstrated a reverse correlation between HLA-G expression and cancer patients' clinical outcome. However, in some studies, HLA-G expression was shown to be associated with a favorable clinical outcome, such as in the case of gastric cancer (Ishigami et al., 2006) and ovarian cancer (Davidson et al., 2005). Others have indicated that HLA-G expression might not have any prognostic value, such as in the case of multiple myeloma (Leleu et al., 2005) and B-CLL (Giannopoulos et al., 2008; Perez-Chacon et al., 2009). The reason behind these contradictory observations remains unclear. Nevertheless, given the notion that HLA-G expression favors tumor development and metastasis in every phase of cancer immunoediting by impairing anti-tumor immune response (Urosevic and Dummer, 2008); aberrant expression of HLA-G in tumors should inevitably result in unfavorable clinical outcomes.

HLA-G expression and tumor-infiltrating lymphocytes (TILs)

The role of the tumor microenvironment has been extensively evaluated in histopathological findings with many studies reporting the presence of immune-cell infiltrates in various neoplasms, called tumor-infiltrating lymphocytes (TILs). The presence of TILs indicates an anti-tumor cellular immune response (Yu and Fu, 2006). In addition, the degree of lymphocytic infiltration and especially the presence of lymphocytes within tumor cell nests has been shown to correlate with a better prognosis in many tumor types, such as ovarian cancer (Zhang et al., 2003), breast cancer (Demaria et al., 2001), melanoma (Piras et al., 2005), esophageal cancer (Schumacher et al., 2001), hereditary nonpolyposis colorectal cancer (Sandel et al., 2005) and endometrial cancer (Kondratiev et al., 2004). Therefore, an examination of the degree of TILs may be used as a favorable prognostic indicator in the histopathology of cancer patients. However, not all the research results are homogeneous as there are studies that relate TILs to a

Table 1. Association of HLA-G expression with clinical outcome in various types of carcinoma.

Type of neoplasia	Sample size	Methods detection	HLA-G expression	Associated with survival	reference
Ovarian carcinoma	148	IHC	33-50%	Favorite prognostic factor	Davidson et al. 2005
Ovarian carcinoma	41	Real time PCR	+55%	Significantly correlated between HLA-G and survival	Jun et al. 2009
		Western blot	++25%		
		IHC	+++20%		
Breast cancer	39	IHC	41%	possible association with shorter disease-free survival	Kleinberg et al, 2006
Breast cancer	235	IHC	66%	Independent prognostic predictor	He et al. 2010
Colorectal cancer	200	IHC	64.6%	Independent prognostic predictor	Ye et al. 2007
Lung cancer	106	IHC	75%	Independent prognostic predictor	Yie et al. 2007
Lung cancer	114	ELISA	sHLA-G exclusively elevated	Both sHLA-G and sHLA-I are independent prognostic predictor	Schütt et al. 2010
Esophageal cancer	121	IHC	90.9%	Independent prognostic predictor	Yie et al. 2007
Gastric cancer	115	IHC		Favorite prognostic factor	Ishigami et al. 2006
Gastric cancer	160	IHC	71%	Independent prognostic predictor	Yie et al. 2007
Hepatocellular carcinoma	180	IHC	+ 43%, +++ 57%	High expression HLA-G independently associated with sort survival and increased recurrence	Cai et al. 2009
Hepatocellular carcinoma	219	IHC	50.2%	strongly correlated to advanced disease stage	Lin et al 2010a,b
Endometrial carcinoma	44	IHC	55%	Correlated with disease stages	Barrier et al. 2006
Endometrial carcinoma	554	IHC	39.8%	MHC-I downregulation is associated with disease prognosis and HLA-G is associated with MHC-I	Bijen et al. 2009
Acute myeloid leukaemia	87	FC	18%	Associated with low T lymphocyte and high leukaemic blast cell	Yan et al. 2008; Nuckel et al. 2005
B-CLL	47	FC	1-54%	Independent prognostic predictor	Erikci et al. 2009
B-CLL	41	ELISA RT-PCR	No change between stages	No additional prognostic value	Glannopoulos et al 2008

worsening outcome in other cancer types (de la Cruz-Merino et al., 2008).

In vitro experiments have demonstrated that HLA-G can induce apoptosis of activated CD8+ lymphocytes and NK cells through mediation of Fas/FasL ligand (Hofmeister and Weiss, 2003; Lindaman et al., 2006). In clinical settings, a reverse correlation between HLA-G expression in tumors and the degree of TILs was demonstrated in CRC (Ye et al., 2007), GC (Yie et al., 2007a), NSCLC (Yie et al., 2007b), ESCC (Yie et al., 2007c) and breast cancer (He et al., 2010) (Fig. 1). A significant correlation between HLA–G expression and TILs score or the counting of CD57-expressing cells was also evident in CIN patients with HPV infection and cervical cancer cases, in which HLA-G expression was significantly higher when compared to CIN patients without HPV infection and pre-malignant lesions (Dong et al., 2010). These results confirm the apoptotic effect of HLA-G on immune cells *in vivo*, and provide evidence of the unfavorable effect associated with HLA-G expression in tumor and patients' clinical outcome.

Furthermore, TILs are not only composed of tumor-specific cytotoxic T lymphocytes and NK cells, but also contain regulatory T cells (T regs) (de la Cruz-Merino et al., 2008). T regs are a subset of immune T cells that inhibit the anti-tumor functions of tumor-specific T cells (Shevach, 2002), and are characterized by the expression of CD4, CD25 and FoxP3 (Hori et al., 2003). Higher levels of T regs in both metastatic lymph nodes and the peripheral blood have been associated with a poor prognosis in several tumor types, while the depletion of CD4+CD25+FoxP3 T cells was found to improve tumor immunity and induce effective tumor rejection (Zou, 2006).

HLA-G5 secreted by mesenchymal stem cells have been found to contribute first to the inhibition of allogeneic T cell proliferation, and then to the expansion of CD4(+)CD25(high)FOXP3(+) regulatory T cells in blocking experiments using neutralizing anti-HLA-G antibody (Selmani et al., 2008). In clinical settings, Chen and colleagues reported that CD4+CD25+FoxP3+ T regs increased markedly in breast cancer patients when compared to normal controls, with the increased frequency of T regs strongly correlated to sHLA-G levels (Chen et al., 2010). However, Cai and colleagues reported that no correlation between HLA-G expression and T regs was found, nor with CD8+ TILs in HCC (Cai et al., 2009). Nevertheless, in their report, a positive correlation was discovered between HLA-G expression in tumor and T regs/CD8+ ratio with patients showing high HLA-G expressions having higher T regs/CD8+ ratios when compared to patients showing low HLA-G expressions. High T regs/CD8+ ratio was in close association with larger tumor size, vascular invasion, elevated AFP levels and advanced stages. In light of this, it was argued that a combination of HLA-G and T regs/CD8+ ratio can serve as a better prognosticator, where patients with concurrent high levels of both variables had more than three times the risk of death and

tumor relapse than those with concurrent low levels.

In all, these data suggest that the estimation of the degree and composition of TILs, as well as HLA-G expression, may be more accurate in assessing cancer patients' clinical outcome and host immune response. This might also explain some of the contradictory observations concerning HLA-G as a prognostic marker in certain studies described above.

HLA-G as a diagnostic marker for cancer patients

Tumor marker is a substance found in the blood, urine, or body tissue, and which has elevated levels in cancer when compared to other tissue types. There have been many different tumor markers used in oncology clinical settings to help detect the presence of cancer with varying degrees of sensitivity and specificity. However, most of the presently used tumor markers are not specific enough for cancer diagnosis because they are also expressed in a variety of normal tissues, benign tumors, and non-neoplastic diseases.

Consequently, there is a strong demand for novel markers with high sensitivity and specificity that produce a low number of false-negative and false-positive results in oncology clinical settings. An ideal biomarker for malignancy will also need to show variation in expression that is associated with the process of neoplastic transformation, and should be early detectable in the pre-malignant phase.

IHC is an important adjunctive test in diagnostic surgical pathology. Usually, the pathologic diagnosis of cancer is not difficult even though it is based on examining the unique morphological features of each tumor type. However, the diagnosis can be challenging at times, especially in final needle aspirates and peritoneal or thoracic washing specimens, where the amount of tumor cells is insufficient for an optimal pathological assessment. In this case, HLA-G IHC staining can be used to distinguish malignant versus benign neoplasms in patients (Shih, 2007) since HLA-G expression is restricted to a few normal tissue types, and is specifically expressed in a variety of neoplastic diseases *in situ*, and is associated with the process of neoplastic transformation (Ye et al., 2007; Yie et al., 2007a-c; Cai et al., 2009; Jung et al., 2009; He et al., 2010).

Noninvasive biomarkers in body fluids can be analyzed rather easily and economically. Therefore, they have the potential to greatly enhance screening and monitoring acceptance. sHLA-G derives from the secretion of soluble isoforms such as HLA-G5, -G6 and -G7, as well as from the shedding of proteolitycally cleaved surface isoforms such as -G1 (Pistoia et al., 2007). sHLA-G can be detected in the blood and other body fluids, such as ascites from healthy individuals utilizing variations of a double determinant immunoassay as a test system. However, a number of studies have reported increased levels of sHLA-G levels in patients with melanoma (Ugurel et al., 2001), acute
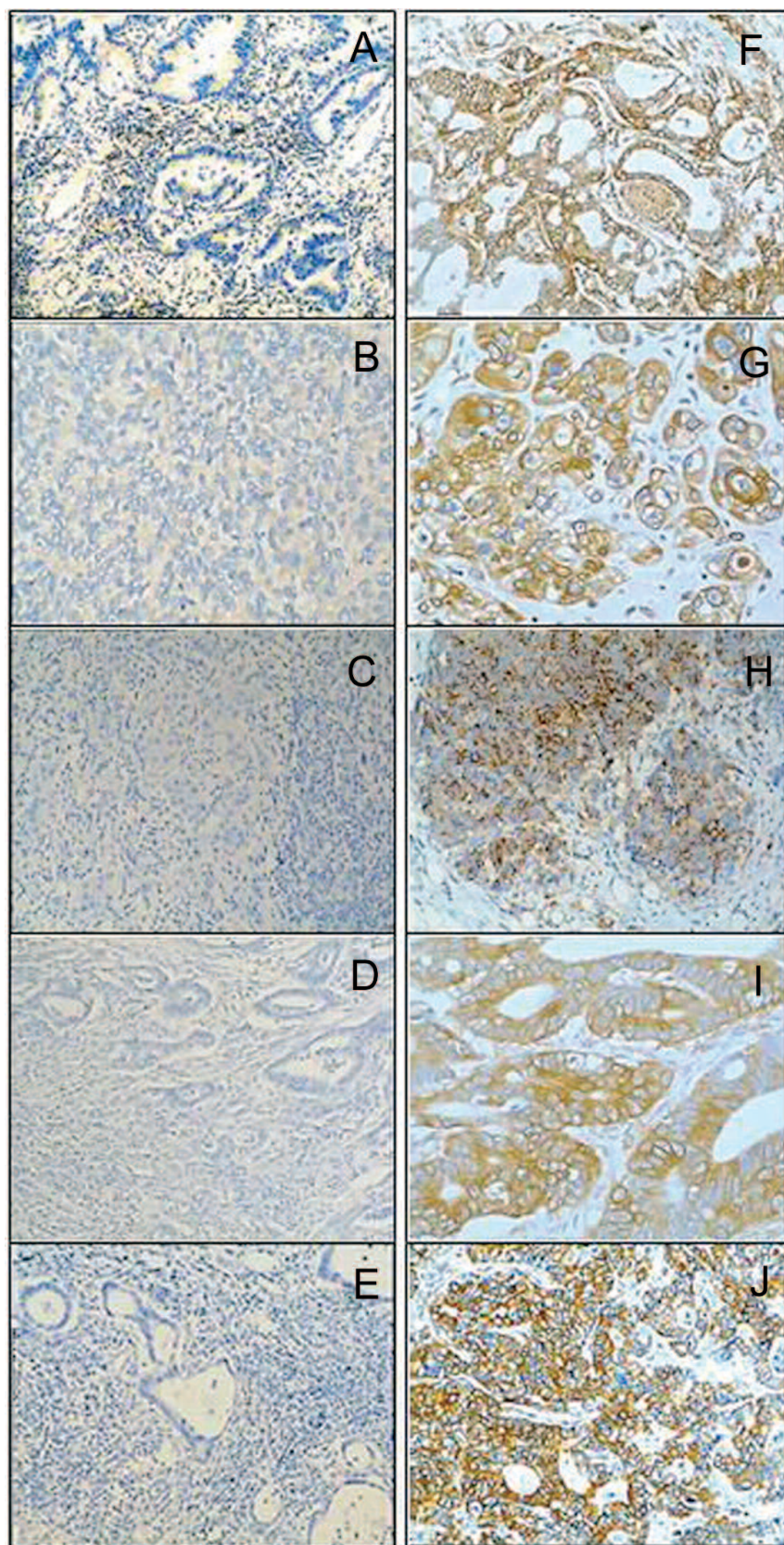
Fig. 1. Reverse Correlation between HLA-G expression and host immune response as estimated by counting the number of TILs. Numerous lymphocytes are evident in tumors with negative HLA-G staining in NSCLC **(A)**, ESCC **(B)**, breast cancer **(C),** CRC **(D)**, and GC **(E)**, while very few lymphocytes are present in tumors with strong HLA-G expression in NSCLC **(F)**, ESCC **(G)**, breast cancer **(H)**, CRC **(I)**, and GC **(J)**. x 200

leukemia (Gros et al., 2006), multiple myeloma (Leleu et al., 2005), neuroblastoma (Morandi et al., 2007), lymphoproliferative disorders (Sebti et al., 2007), and breast or ovarian cancer (Singer et al., 2003) when compared to healthy controls or benign subjects. These reports provide preliminary evidence that suggests sHLA-G can be used as a potential biomarker in body fluids for the diagnosis of cancer.

The receiver operating characteristic (ROC) curve is a tool commonly used to evaluate biomarker utility in the clinical diagnosis of disease, especially during biomarker development research (Perkins et al., 2009). Singer and colleagues applied a sensitive ELISA to measure sHLA-G levels in the supernatant of peritoneal ascites from ovarian and breast cancer patients and benign control subjects (Singer et al., 2003). In their study, the levels of sHLA-G were significantly higher in malignant ascites when compared to benign ascites. In order to detect ovarian and breast cancer using multiple cutoff values, the investigators used ROC curves to evaluate the performance of sHLA-G. The area under the ROC curve was determined to be 0.95 in assessing sHLA-G levels as a diagnostic tool. Two other research groups obtained similar results when they measured and compared sHLA-G levels in plasma specimens from breast cancer patients and healthy donors (Chen et al., 2010; He et al., 2010). In these studies, the areas under the ROC curves were reported to be 0.953 and 0.98, respectively.

In our own research work, we also analyzed sHLA-G in plasma from early stage of patients with NSCLC, ESCC, CRC and GC and found that sHLA-G levels increased dramatically in cancer patients when compared to normal healthy controls. The areas under the ROC curves were given as 0.97, 0.91, 0.98 and 0.80 for healthy controls versus CRC, GC, ESCC and NSCLC, respectively. Given 100% specificity at a cutoff value, the highest detection sensitivity achieved was 94% (95% CI: 89-99) for CRC, 85% (95% CI: 76-94) for GC, 91% (95% CI: 88-94) for ESCC, and 51% (95% CI: 43-59) for NSCLC (see Fig. 2).

In a recent study of colorectal cancer, Zhu and colleagues measured sHLA-G levels in serum from cancer patients and patients with benign colorectal disease (Zhu et al., 2010). They found that the median sHLA-G concentration was significantly higher in colorectal cancer when compared to normal colorectum, hyperplastic polyp, inflammatory bowel disease, and adenoma. The area under the ROC curve was found to be 0.84 with a sensitivity of 72.2% and a specificity of 87.8%. They also discovered that sHLA-G was superior to carcinoembryonic antigen (CEA) in terms of differentiating colorectal cancer from benign colorectal diseases, and the combination of sHLA-G and CEA showed a higher detection capacity than using the individual markers separately.

Table 2 summarizes the available HLA-G data as a potential biomarker in the diagnosis of human carcinomas. Nevertheless, the data is still limited, and it

has to be recognized that serum/plasma sHLA-G levels can be changed with various physiological and pathological factors such as pregnancy, organ transplantation, autoimmune disease or viral infection (Pistoia et al., 2007). Thus, the use of sHLA-G concentration in body fluids as a biomarker in the diagnosis and management of malignancies needs to be explored further. As well, research into how other clinical status might affect HLA-G levels in serum/plasma is necessary before any final application to cancer diagnosis. For that reason, it will be important to carry out analyses on a large number of samples in multiple centers.
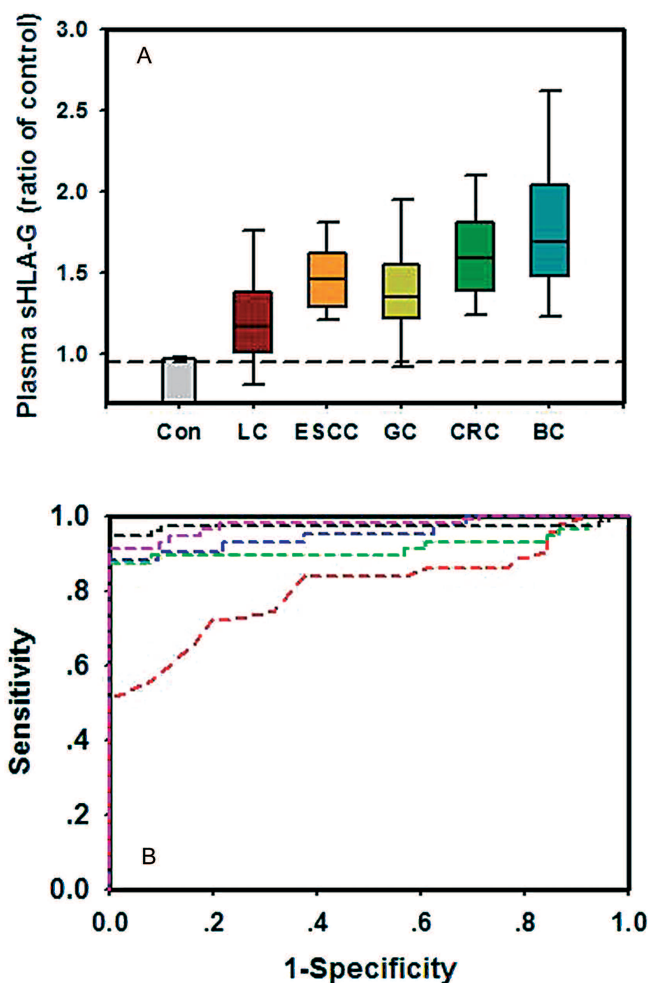

Fig. 2. **A.** sHLA-G levels in plasma samples in patients with early stages of breast cancer, NSCLC, ESCC, CRC, and GC were compared with normal healthy controls. A cutoff value was determined when specificity was set to 100% according to ROC curve analysis. **B.** ROC curve analyses of cancer patients versus healthy controls used to assess the significance of diagnosis by measuring plasma sHLA-G levels in breast cancer (---), NSCLC (---), ESCC (---), CRC (---) and GC (---). Areas under the ROC curves are 0.95 for breast cancer, 0.97 for CRC, 0.91 for GC, 0.98 for ESCC, and 0.80 for NSCLC, respectively.

Regardless, HLA-G expression has a high specificity for malignancies. It also has a relatively high prevail rate in all the common types of malignancies examined, even though it is not a special marker for any one type of cancer. Consequently, the detection of sHLA-G in serum/plasma may be used for screening common types of cancers on a population. Moreover, a panel consisting of HLA-G and certain specific tissue markers may be able to not only identify cancer patients from normal individuals, but also distinguish patients with specific types of cancer.

HLA-G as an attractive target for tumor immunotherapy

The main premise of cancer immunotherapy is to restore or further stimulate the patient's immune system in order to eliminate malignant tumor cells. This can be achieved through either cancer vaccines or the administration of drugs consisting of antagonistic recombinant proteins or neutralizing antibodies to block immunoinhibiting pathways.

As we know, HLA-G plays an important role in tumor immune escape mechanisms. Since its expression in tumor tissues is a unique feature that is highly specific for malignant transformation and negatively correlates with clinical outcomes, the molecule is considered an attractive target for cancer immunotherapy. Many approaches have been proposed to use HLA-G as a cancer immunotherapeutic target. These include revisiting HLA-G expression in tumor cells; intervening inhibitory pathways; and using antibody delivery methods to bring cytotoxic drugs to more invasive and metastatic cancer cells.

With regards to revisiting HLA-G expression in tumor cells, several anti-cancer drugs have been reported to be inducers for cancer cells that express higher levels of HLA-G proteins, resulting in tumor evasion of the host immune system. For example, 5-aza-2'-deoxycytidine, a demethylating agent for treating cancer

patients during epigenetic therapy could reactivate HLA-G protein expression in many cell lines tested (Poláková et al., 2009). Similarly, IFN immunotherapy of malignant tumors can cause immune invasion side effects by up-regulating the expression of HLA-G at tumor sites (Mitsdoerffer et al., 2004). Therefore, it appears that revisiting HLA-G expression in cancer patients is very important when considering immuno-therapeutic strategies, such as immunization, which is currently being used to generate tumor antigen-specific cytotoxic T cells, IFN administration, or chemotherapy with DNA demethylating drugs in which the up-regulation of HLA-G expression may constitute an adverse effect.

In order to block HLA-G functional pathways, overcoming the suppressive activity of HLA-G–related regulatory T cells, or selectively depleting these cells locally in tumors or draining lymph nodes, may represent important therapeutic strategies aimed at inducing effective tumor-specific immune responses capable of controlling and eradicating tumors (Curiel, 2007; Ballimore and Golkin 2008; Carosella et al., 2008b).

Since the main inhibitory effects of HLA-G are mediated by its interaction with lymphocyte immunoglobulin-like receptors, blocking the reaction by antagonistic recombinant proteins or neutralizing antibodies should be beneficial in treating HLA-G-expressing cancer cells. For example, by using *in vitro* allostimulation assays, antibodies against LIR-1 and LIR-2 have successfully restored T cell proliferation (Clements et al., 2007). Similar results have been obtained when treating cancer cells with anti-FasL antibodies that block the Fas/FasL pathway based on the idea that secreted HLA-G5 can bind to CD8 and induce Fas/Fas ligand-mediated apoptosis in activated CD8+ lymphocytes (Hofmeister and Weiss, 2003).

Furthermore, direct application of HLA-G neutralizing antibodies may minimize the inhibitory effects of HLA-G molecules. As such, our own research

Table 2. HLA-G as a potential tumor marker for diagnosis in human carcinoma

Type of neoplasia	Specimen	Assay Methods	Comparison	ROC curve area	Specificity %	Sensitivity%	References
CRC	Sera	ELISA		0.842	87.8	72.2	Zhu et al 2010
CRC	Plasma	ELISA	37 p* vs. 260 c**	0.94	100	94	Cao et al (unpublished data)
Breast cancer	Ascetics	ELISA	17 p vs. 19 b***	0.90	100	65	Singer et al, 2003
Breast cancer	Plasma	ELISA	44 p vs. 48 c	0.95	100	88.1	He et al, 2010
Breast cancer	Plasma	ELISA	92p vs. 70 c	0.95			Chen et al 2010
Ovarian cancer	Ascetics	ELISA	24 p vs. 19 b	0.99	100	84	Singer et al, 2003
Ovarian cancer	Tissue	IHC/western blot					Jung et al, 2009
Renal carcinoma	Sera	ELISA	16 p vs. 144 c	0.733			Li et al, 2009
ESCC	Plasma	ELISA	58 p vs. 260 c	0.98	100	91	Cao et al (unpublished data)
NSCLC	Plasma	ELISA	43 p vs. 260 c	0.80	100	51	Cao et al (unpublished data)
Gastric cancer	Plasma	ELISA	18 p vs. 160 c	0.91	100	85	Cao et al (unpublished data)
Endometrial carcinoma	Tissue	IHC		0.75			Barrier et al. 2006

* p: patients; **c: healthy control; *** b: benign tumor

has shown that allo-CTL response *in vitro* to MCF-7 breast cells that have positive HLA-G expression was restored or further strengthened by the addition of anti-HLA-G antibodies in a cytotoxicity study (He et al., 2010). Similar results showed that HLA-G expression dramatically inhibits cell lyses by NK-92 cells, which could then be restored by the anti-HLA-G conformational mAb in ovarian cancer cell lines (Lin et al., 2007).

A target-based chemotherapy can be achieved by employing HLA-G antibody delivery methods to bring cytotoxic drugs to tumor tissues, since HLA-G is specifically expressed in the majority of cases of various common types of cancer, and is presented as either a membrane-associated or membrane/cytoplasmic pattern. Small interfering RNA/small hairpin RNA therapies can also be employed to arbitrate HLA-G-expressing cells similar to the antibody-oriented route. Also, anti-HLA-G antibodies could be utilized for developing a cancer imaging system to monitor the activity and location of tumor cells that have been tolerated by the immune defense system.

Nevertheless, all hypotheses and recommended methods based upon *in vitro* experimental results have to be verified in animal experiments and clinical trials in order to evaluate their efficacy in clinical applications. In addition, HLA-G antibodies for a target-based chemotherapy/radiotherapy have to be aware that recent research has shown that MSC expresses HLA-G (Favier et al., 2007). Cytotoxic drugs conjugated HLA-G anibodies might generate side-inhibitory effects on the bone marrow stem cells of cancer patients.

HLA-G and other cancer immunoediting markers

During the last decade, many approaches have been developed to evaluate novel blood-based biomarkers for the diagnosis of cancer. According to recent reviews, some tests may offer the potential to improve current tumor markers in the diagnosis, prognosis and management of cancer (Hundt et al., 2007; Jotwani and Gralow, 2009; Gagnon and Ye, 2008, Greenberg and Lee, 2007; Anderson et al., 2006).

Overall, most of the new tumor markers are associated with uniqueness of the tumor cells themselves. However, as described above, disease progression in cancer patients is not solely determined by the characteristics of the tumor, but also by the host immune response. Since tumor immunoediting mechanisms play a critical role in cancer development, progress, metastasis and recurrence, it is valuable to identify specific tumor markers for tumor immune escape for use in clinical settings.

Many factors in the tumor microenviroment have been shown to contribute tumor escape, such as the expression of HLA-G and indoleamine 2,3-dioxygenase (IDO), the down-regulation of MHC molecules in tumors, the presence of CD4+CD25+ FaxP3+T regs, and the expansion of immunosuppressive myeloid cell populations (Swann and Smyth, 2007). Tumor resistance to cytotoxic pathways, as observed in tumors with mutations genes encoding FAS and TRAIL death receptor 5 (DR5), and in the overexpression of antiapoptotic molecules FLIP and BCL-XL are involved as well (Swann and Smyth, 2007). Theoretically, all of these factors could be used as biomarkers for malignant disease and host immune status, but available data suggests that IDO and Fas/FasL may have clinical applications in addition to HLA-G.

Studies show that the detection of IDO expression may be used as a prognosis indicator in patients with renal cell carcinoma (Riesenberg et al., 2007), melanoma (Weinlich et al., 2007), endometrial cancer (Ino et al., 2006), colorectal cancer (Brandacher et al., 2006) and hepatocellular carcinoma (Pan et al., 2008). Also, it has been suggested that soluble FasL might serve as a prognostic or diagnostic marker in melanoma (Uqurel et al., 2001; Neuber and Eidam, 2006), lung cancer (Shimizu et al., 2005; Fokkema et al., 2006), colorectal cancer (Nadal et al., 2005), cervical cancer (Munakata et al., 2005), glioma (Strege et al., 2004) and breast cancer (Reimer et al., 2002).

Moreover, HLA-G and IDO display common properties in terms of expression, function, and regulation (Gonzalez-Hernandez et al., 2005). Also, the Fas/FasL pathway is recruited by HLA-G to exert its immunosuppressive function on both T cells (Naji et al., 2007) and angiogenesis (Fons et al., 2006). However, when compared to HLA-G, both IDO and Fas/FasL systems are also expressed in a wide range of normal tissues (Leithauser et al., 1993; Dai and Zhu, 2010). From this standpoint, IDO and Fas/FasL may not be as specific as HLA-G for malignant detection, although all the molecules have been shown to play crucial immunosuppressive roles in tumor immunoediting mechanisms.

Conclusions

One of the main goals in the current research of HLA-G is applying its use in the clinic, either for diagnostic/prognostic applications or as a therapeutic tool/target. There has been persuasive evidence from the studies cited in this review that suggests HLA-G is a potential biomarker for the diagnosis and prognosis of various human cancers. This novel tumor marker differs from other currently used tumor markers by the following two unique characteristics: 1) it is expressed in transformed malignant cells but not in normal or benign tissues; and 2) it is functionally involved in tumor escape mechanisms. Given these characteristics, HLA-G is an attractive target for developing new interventions to restore host immune response against tumor cells, or can be used in antibody delivery methods to bring cytotoxic drugs to cancer cells as in the case of cancer immunotherapy.

However, the use of sHLA-G as a diagnostic or screening marker for human cancer needs to be explored

further. More investigations on how other clinical status might affect HLA-G levels in serum/plasma should be performed. Additional analyses on a large number of samples in multiple centers should be done before any final application in oncology clinical settings. Moreover, most of the current emphasis is being placed on HLA-G as diagnostic and prognostic biomarker, but it is equally likely that HLA-G can be utilized to monitor therapeutic responses (Davidson et al., 2005). In patients where HLA-G expression has been detected, changes in levels of the molecule might reflect changes in tumor and host immune status.

References

Anderson C., Nijagal A. and Kim J. (2006). Molecular markers for gastric adenocarcinoma: an update. Mol. Diagn. Ther. 10, 345-352.

Ballimore A. and Golkin A. (2008). Regulatory T cells and tumour immunity: observations in mice and men. Immunology 123, 157-163.

Barrier B.F., Kendall B.S., Sharpe-Timms K. and Kost E.R. (2006). Characterization of human leukocyte antigen-G (HLA-G) expression in endometrial adenocarcinoma. Gynecol. Oncol. 103, 25-30.

Bijen C.B., Bantema-Joppe E.J., de Jong R.A., Leffers N., Mourits M.J., Eggink H.F., van der Zee AG., Hollema H., de Bock G.H and Nijman H.W. (2010). The prognostic role of classical and nonclassical MHC class I expression in endometrial cancer. Int. J. Cancer 126, 1417-1427.

Brandacher G., Perathoner A., Ladurner R., Schneeberger S., Obrist P., Winkler C., Werner E-R., Werner-Felmayer G., Weiss H.G., Göbel G., Margreiter R., Königsrainer A., Fuchs D and Amberger A. (2006). Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. Clin. Cancer Res. 12, 1144-1151.

Cai M.Y., Xu Y.F., Qiu S.J., Ju M.J., Gao Q., Li Y.W., Zhang B.H., Zhou J. and Fan J. (2009). Human leukocyte antigen-G protein expression is an unfavorable prognostic predictor of hepatocellular carcinoma following curative resection. Clin. Cancer Res. 15, 4686-4693.

Carosella E.D., Moreau P., Lemaoult J. and Rouas-Freiss N. (2008a). HLA-G: from biology to clinical benefits. Trends Immunol. 29, 125-132.

Carosella E.D., Benoit Favier B., Rouas-Freiss N., Moreau P. and Joel LeMaoult J. (2008b). Beyond the increasing complexity of the immunomodulatory HLA-G molecule. Blood 111, 4862-4870.

Chen H.X., Lin A., Shen C.J., Zhen R., Chen B.G., Zhang X., Cao F.L., Zhang J.G and Yan W.H. (2010). Up-regulation of human leukocyte antigen-G (HLA-G) expression and its clinical significance in ductal breast cancer. Hum. Immunol. 71, 892-898.

Cirulli V., Zalatan J., McMaster M., Prinsen R., Salomon DR., Ricordi C,. Torbett B.E, Meda P. and Crisa L. (2006). The class I HLA repertoire of pancreatic islets comprises the nonclassical class Ib antigen HLA-G. Diabetes 55, 1214–1222.

Clements C.S., Kjer-Nielsen L., McCluskey J. and Rossjohn J. (2007). Structural studies on HLA-G: implications for ligand and receptor binding. Hum. Immunol. 68, 220-226.

Crisa L., McMaster M.T., Ishii J.K., Fisher S.J. and Salomon D.R. (1997). Identification of a thymic epithelial cell subset sharing expression of the class Ib HLA-G molecule with fetal trophoblasts. J. Exp. Med. 186, 289-298.

Curiel T. (2007). Tregs and rethinking cancer immunotherapy. J. Clin. Invest. 117, 1167-1174.

Dai X.C. and Zhu B.T. (2010). Indoleamine 2,3-dioxygenase tissue distribution and cellular localization in mice: Implications for its biological functions. J. histochem. Cytochem. 58, 17-28,

Davidson B., Elstrand M.B., McMaster M.T., Berner A., Kurman R.J., Risberg B., Trope C.G. and Shih IeM. (2005). HLA-G expression in effusions is a possible marker of tumour susceptibility to chemotherapy in ovarian carcinoma. Gynecol. Oncol 96, 42-47.

de la Cruz-Merino L., Grande-Pulido E., Albero-Tamarit A and Codes-Manuel de Villena M.E. (2008). Cancer and immune response: old and new evidence for future challenges. Oncologist 13, 1246-1254.

Demaria S., Volm M.D., Shapiro R.L., Yee H.T., Oratz R., Formenti S.C., Muggia F. and Symmans W.F. (2001). Development of tumor-infiltrating lymphocytes in breast cancer after neoadjuvant paclitaxel chemotherapy. Clin. Cancer Res. 7, 3025-3030.

Dong D.D., Yang H., Li K., Xu G., Song L.H., Fan X.L., Jiang X.L and Yie S.M. (2010). Human Leukocyte Antigen-G (HLA-G) expression in cervical lesions: Association with cancer progression, HPV 16/18 infection, and host limune response. Reprod. Sci. 17, 718-723.

Dunn G.P., Bruce A.T., Ikeda H., Old L.J. and Schreibe R.D. (2002). Cancer immunoediting: from immunosurveillance to tumor escape. Nat. Immunol. 3, 991-998.

Dunn G.P., Old L.J. and Schreiber R.D. (2004). The three Es of cancer immunoediting. Annu. Rev. Immunol. 22, 329-360.

Erikci A.A., Karagoz B., Ozyurt M., Ozturk A., Kilic S. and Bilgi O. (2009). HLA-G expression in B chronic lymphocytic leukemia: a new prognostic marker? Hematology 14, 101-105.

Favier B., LeMaoult J., Rouas-Freiss N., Moreau P., Menier C. and Carosella E.D. (2007). Research on HLA-G: an update. Tissue Antigens 69, 207-211.

Fokkema E., Timens W., de Vries E.G., de Jong S., Fidler V., Meijer C. and Groen H.J. (2006).Expression and prognostic implications of apoptosis-related proteins in locally unresectable non-small cell lung cancers. Lung Cancer 52, 241-247.

Fons P., Chabot S., Cartwright J.E., Lenfant F., L'Faqihi F., Giustiniani J and Jean-Pascal Herault J.P. (2006).Soluble HLA-G1 inhibits angiogenesis through an apoptotic pathway and by direct binding to CD160 receptor expressed by endothelial cells. Blood 108, 2608-2615.

Gagnon A and Ye B. (2008). Discovery and application of protein biomarkers for ovarian cancer. Curr. Opin. Obstet. Gynecol. 20, 9-13.

Giannopoulos K., Schmitt M., Kowal M., Własiuk P., Bojarska-Junak A., Roliński J and Dmoszyńska A. (2008). The significance of soluble HLA-G plasma levels as well as messenger HLA-G for B-cell chronic lymphocytic leukemia (B-CLL). Leuk. Res. 32, 1815-1819.

Gonzalez-Hernandez A., Lemaoult J., Lopez A., Alegre E., Caumartin J., Le Rond S., Daouya M., Moreau P. and Carosella E.D. (2005). Linking two immuno-suppressive molecules: indoleamine 2,3 dioxygenase can modify HLA-G cell-surface expression. Biol. Reprod. 3, 571-578.

Greenberg A.K. and Lee M.S.(2007). Biomarkers for lung cancer: clinical uses. Curr. Opin. Pulm. Med. 13, 249-255.

Gros F., Sebti Y., de Guibert S., Branger B., Bernard M., Fauchet R. and Amiot L. (2006). Soluble HLA-G molecules increase during acute leukemia, especially in subtypes affecting monocytic and lymphoid lineages. Neoplasia 8, 223-230.

He X., Dong D.D., Yie S.M., Yang H., Cao M., Ye S.R., Li K., Liu J. and Chen J. (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, 1459-1469.

Hofmeister V. and Weiss E.H. (2003). HLA-G modulates immune responses by diverse receptor interactions. Semin. Cancer Biol. 13, 317-323.

Hori S., Nomura T. and Sakaguchi S. (2003). Control of regulatory T cell development by the transcription factor FoxP3. Science 299, 1057-1061.

Hundt S., Haug U. and Brenner H. (2007). Blood markers for early detection of colorectal cancer: a systematic review. Cancer Epidemiol. Biomarkers Prev. 16, 1935-1953.

Ino K., Yoshida N., Kajiyama H., Shibata K., Yamamoto E., Kidokoro K., Takahashi N., Terauchi M., Nawa A., Nomura S., Nagasaka T., Takikawa O. and Kikkawa F. (2006). Indoleamine 2,3-dioxygenase is a novel prognostic indicator for endometrial cancer. Br. J. Cancer. 95, 1555-1561.

Ishigami S., Natsugoe S., Miyazono F., Nakajo A., Tokuda K., Matsumoto M., Okumura H., Douchi T., Hokita S and Aikou T. (2006). HLA-G expression in gastric cancer. Anticancer Res. 26, 2467-2472.

Jotwani A.C and Gralow J.R. (2009). Early detection of breast cancer: new biomarker tests on the horizon? Mol. Diagn. Ther. 13, 349-357.

Jung Y.W., Kim Y.T., Kim S.W., Kim S., Kim J.H., Cho N.H. and Kim J.W. (2009). Correlation of human leukocyte antigen-G (HLA-G) expression and disease progression in epithelial ovarian cancer. Reprod. Sci. 16, 1103-1111.

Kleinberg L., Flørenes V.A., Skrede M., Dong H.P., Nielsen S., McMaster M.T., Nesland J.M., Shih IeM and Davidson B. (2006). Expression of HLA-G in malignant mesothelioma and clinically aggressive breast carcinoma. Virchows Arch. 449, 31-39.

Kondratiev S., Sabo E., Yakirevich E., Lavie O. and Resnick M.B. (2004). Intratumoral CD8+ T lymphocytes as a prognostic factor of survival in endometrial carcinoma. Clin. Cancer Res. 10, 4450–4456.

Laheru D. and Jafee E. (2007). The cancer handbook. Cancer vaccines. Second Edition. Chichester, UK, Wiley. pp 1268-1274.

Le Discorde M., Moreau P., Sabatier P., Legeais J.M. and Carosella E.D. (2003). Expression of HLA-G in human cornea, an immune-privileged tissue. Hum. Immunol. 64, 1039-1044.

Leithauser F., Dhein J., Mechtersheimer G., Koretz K., Bruderlein S., Henne C., Schmidt A., Debatin K.M., Krammer P.H and Moller P. (1993). Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily, in normal and neoplastic cells. Lab. Invest. 69, 415-429

Leleu X., Le Friec G., Facon T., Amiot L., Fauchet R., Hennache B., Coiteux V., Yakoub-Agha I., Sylvain Dubucquoi S. Avet-Loiseau H., Mathiot C., Bataille R., Jean-Yves Mary J.Y. and on behalf of the Intergroupe Francophone du Myélome. (2005). Total soluble HLA class I and soluble HLA-G in multiple myeloma and monoclonal gammopathy of undetermined significance. Clin. Cancer Res. 11, 7297-7303.

Li B.L., Lin A., Zhang X.J., Zhang X., Zhang J.G., Wang Q., Zhou W.J., Chen H.X., Wang T.J. and Yan W.H. (2009). Characterization of HLA-G expression in renal cell carcinoma. Tissue Antigens 74, 213-221.

Lin A., Yan W.H., Xu H.H., Gan M.F., Cai J.F., Zhu M. and Zhou M.Y. (2007). HLA-G expression in human ovarian carcinoma counteracts NK cell function. Ann. Oncol. 18, 1804-1809.

Lin A., Chen H.X., Zhu C.C., Zhang X., Xu H.H., Zhang J.G., Wang Q., Zhou W.J and Yan W.H. (2010a). Aberrant human leukocyte antigen-G expression and its clinical relevance in hepatocellular carcinoma. J. Cell Mol. Med. 14, 2162-2171.

Lin A., Zhu C.C., Chen H.X., Chen B.F., Zhang X., Zhang J.G., Wang Q., Zhou W.J., Hu W., Yang H.H., Xu H.H and Yan W.H.(2010b). Clinical relevance and functional implications for human leukocyte antigen-G expression in non-small-cell lung cancer. J. Cell Mol. Med. 14, 2318-2329.

Lindaman A., Dowden A. and Zavazava N. (2006). Soluble HLA-G molecules induce apoptosis in natural killer cells. Am. J. Reprod. Immunol. 56, 68-76.

Menier C., Saez B., Horejsi V., Martinozzi S., Krawice-Radanne I., Bruel S, Le Danff C., Reboul M., Ivan Hilgert I., Rabreau M, Larrad M.L., Pla M., Carosella E.D. and Rouas-Freiss N. (2003). Characterization of monoclonal antibodies recognizing HLA-G or HLA-E: new tools to analyze the expression of nonclassical HLA class I molecules. Hum. Immunol 64, 315-326.

Mitsdoerffer M., Schreiner B., Kieseier B.C., Neuhaus O., Dichgans J., Hartung H.P., Weller M. and Wiendl H. (2004). Monocyte-derived HLA-G acts as a strong inhibitor of autologous CD4 T cell activation and is upregulated by interferon-ß *in vitro* and *in vivo*: rationale for the therapy of multiple sclerosis. J. Neuroimmunol. 159, 159-164.

Morandi F., Levreri I., Bocca P., Galleni B., Raffaghello L., Ferrone S., Prigione I. and Pistoia V. (2007). Human neuroblastoma cells trigger an immunosuppressive program in monocytes by stimulating soluble HLA-G release. Cancer Res. 67, 6433-6441.

Munakata S., Watanabe O., Ohashi K. and Morino H. (2005). Expression of Fas ligand and bcl-2 in cervical carcinoma and their prognostic significance. Am. J. Clin. Pathol. 123, 879-885.

Nadal C., Maurel J., Gallego R., Castells A., Longarón R., Marmol M., Sanz S., Molina R., Martin-Richard M. and Gascón P.(2005). FAS/FAS ligand ratio: a marker of oxaliplatin-based intrinsic and acquired resistance in advanced colorectal cancer. Clin. Cancer Res. 11, 4770-4774.

Naji A., Durrbach A., Carosella E.l.D. and Rouas-Freiss N. (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, 233-239.

Neuber K. and Eidam N. (2006). Expression of Fas ligand (CD95L) in primary malignant melanoma and melanoma metastases is associated with overall survival. Onkologie 29, 361-365.

Nuckel H., Rebmann V., Durig J., Duhrsen U and Grosse-Wilde H. (2005). HLA-G expression is associated with an unfavorable outcome and immunodeficiency in chronic lymphocytic leukemia. Blood 105, 1694-1698.

Pan K., Wang H., Chen M.S., Zhang H.K., Weng D.S., Zhou J., Huang W., Li J.J., Song H.F and Xia J.C. (2008). Expression and prognosis role of indoleamine 2,3-dioxygenase in hepatocellular carcinoma. J. Cancer. Res. Clin. Oncol. 134, 1247-1253.

Paul P., Rouas-Freiss N., Khalil-Daher I., Moreau P., Beatrice Riteau B., Le Gal F.A., Avril M.F., Dausset J., Guillet J.G. and Carosella E.D. (1998). HLA-G expression in melanoma: a way for tumor cells to escape from immunosurveillance. Proc. Natl. Acad. Sci. USA 95, 4510-4515.

Perez-Chacon G., Rosado S., Rebolleda N., Losada-Fernandez I., Vargas J.A., Morado M., Jorda J. and Perez-Aciego P. (2009). Prognostic irrelevance of HLA-G in B-cell chronic lymphocytic leukemia. Int. J. Lab. Hematol. 31, 327-37.

Perkins N.J., Schisterman E.F. and Vexler A. (2009). Generalized ROC curve inference for a biomarker subject to a limit of detection and masurement error. Stat Med. 28, 1841-1860.

Piras F., Colombari R., Minerba L., Murtas D., Floris C., Maxia C., Corbu A., Perra M.T. and Sirigu P. (2005). The predictive value of CD8, CD4, CD68, and human leukocyte antigen-D-related cells in the prognosis of cutaneous malignant melanoma with vertical growth phase. Cancer 104, 1246-1254.

Pistoia V., Morandi F.,Wang X. and Ferrone S. (2007). Soluble HLA-G: Are they clinically relevant? Semin. Cancer Biol. 17, 469-79.

Poláková K., Bandzuchová E., Kuba D. and Russ G. (2009). Demethylating agent 5-aza-2'-deoxycytidine activates HLA-G expression in human leukemia cell lines. Leuk. Res. 33, 518-24.

Reimer T., Koczan D., Müller H., Friese K., Thiesen H.J. and Gerber B. (2002).Tumour Fas ligand:Fas ratio greater than 1 is an independent marker of relative resistance to tamoxifen therapy in hormone receptor positive breast cancer. Breast Cancer Res. 4, R9.

Riesenberg R., Weiler C., Spring O., Eder M., Buchner A., Popp T., Castro M., Kammerer R., Takikawa O., Hatz R.A., Stief C.G., Hofstetter A. and Zimmermann W. (2007). Expression of indoleamine 2,3-dioxygenase in tumor endothelial cells correlates with long-term survival of patients with renal cell carcinoma. Clin. Cancer Res. 13, 6993-7002.

Sandel M.H., Dadabayev A.R., Menon A.G., Morreau H., Melief C.J.M., Offringa R., van der Burg S.H., Janssen-van Rhijn C.M., Ensink N.G., Tollenaar R.A.E.M., van deVelde C.J.H. and Kuppen P.J.K. (2005). Prognostic value of tumor-infiltrating dendritic cells in colorectal cancer: Role of maturation status and intratumoral localization. Clin. Cancer Res. 11, 2576-2582.

Schumacher K., Haensch W., Röefzaad C. and Schlag P.M. (2001). Prognostic significance of activated CD8+ T cell infiltrations within esophageal carcinomas. Cancer Res. 61, 3932–3936.

Schütt P., Schütt B., Switala M., Bauer S., Stamatis G., Opalka B., Eberhardt W., Schuler M., Horn P.A and Rebmann V. (2010). Prognostic relevance of soluble human leukocyte antigen-G and total human leukocyte antigen class I molecules in lung cancer patients. Hum. Immunol. 2010 71, 489-495.

Sebti Y., Le Maux A., Gros F., De Guibert S., Pangault C., Rouas-Freiss N., Bernard M and Amiot L. (2007). Expression of functional soluble human leukocyte antigen-G molecules in lymphoproliferative disorders. Br. J. Haematol 138, 202-212.

Selmani Z., Naji A., Zidi I., Favier B., Gaiffe E., Obert L., Borg C., Saas P., Tiberghien P., Rouas-Freiss N., Carosella E.D and Deschaseaux F. (2008). Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells. 26, 212-222.

Selmani Z., Naji A., Gaiffe E., Obert L., Tiberghien P., Rouas-Freiss N., Carosella E.D. and Deschaseaux F. (2009). HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. Transplantation 87 (Suppl), S62-66.

Shevach E.M. (2002). CD4+ CD25+ suppressor T cells: More questions than answers. Nat. Rev. Immunol. 2, 389-400.

Singer G., Rebmann V., Chen Y.C., Liu H.T., Ali S.Z., Beinsberg J.,

McMaster M.T., Pfeiffer K., Chan D.W., Wardelmann E., Grosse-Wilde H., Cheng C.C., Kurman R.J. and Shih IeM. (2003).HLA-G is a potential tumor marker in malignant ascites. Clin. Cancer Res. 9, 4460-4464.

Shih I.M. (2007). Application of HLA-G Expression in the Diagnosis of Human Cancer. Hum. Immunol. 68, 272–276.

Shimizu M., Kondo M., Ito Y., Kume H., Suzuki R and Yamaki K. (2005). Soluble Fas and Fas ligand provide new information on metastasis and response to chemotherapy in SCLC patients. Cancer Detect. Prev. 29, 175-180.

Strege R.J., Godt C., Stark A.M., Hugo H.H. and Mehdorn HM. (2004). Protein expression of Fas, Fas ligand, Bcl-2 and TGFbeta2 and correlation with survival in initial and recurrent human gliomas. J. Neurooncol. 67, 29-39.

Swann J.B and Smyth M.J. (2007). Immune surveillance of tumors. J. Clin. Invest. 117, 1137-1146.

Urosevic M. and Dummer R. (2008). Human leukocyte antigen-G and cancer immunoediting. Cancer Res. 68, 627-630.

Ugurel S., Rappl G., Tilgen W. and Reinhold U. (2001). Increased soluble CD95 (sFas/CD95) serum level correlates with poor prognosis in melanoma patients. Clin. Cancer Res. 7, 1282-1286.

Ugurel S., Rebmann V., Ferrone S., Tilgen W., Grosse-Wilde H. and Reinhold U. (2001). Soluble human leukocyte antigen-G serum levelis elevated in melanoma patients and is further increased by interferon-alpha immunotherapy. Cancer 92, 369-376.

Urosevic M., Kurrer M.O., Kamarashev J., Mueller B., Weder W., Burg G., Stahel R.A., Dummer R. and Trojan A. (2001).Human leukocyte antigen G up-regulation in lung cancer associates with high-grade, human histology, leukocyte antigen class I loss and interleukin-10 production. Am. J. Pathol. 159, 817- 824.

Weinlich G., Murr C., Richardsen L., Winkler C. and Fuchs D. (2007). Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. Dermatology. 214, 8-14.

Yan W.H., Lin A., Chen B.G., Luo W.D., Dai M.Z., Chen X.J., Xu H.H. and Li B.L. (2008). Unfavourable clinical implications for HLA-G expression in acute myeloid leukaemia. J. Cell Mol. Med. 12, 889-898.

Ye S.R., Yang H., Li K., Dong D.D., Lin X.M. and Yie S.M. (2007). Human leukocyte antigen G expression: as a significant prognostic indicator for patients with colorectal cancer. Mod. Pathol. 20, 375-383.

Yie S.M., Yang H., Ye S.R., Li K., Dong D.D. and Lin X.M. (2007a). Expression of human leukocyte antigen G (HLA-G) correlates with poor prognosis in gastric carcinoma. Ann. Surg. Oncol. 14, 2721-2729.

Yie S.M., Yang H., Ye S.R., Li K., Dong D.D. and Lin X.M. (2007b). Expression of human leucocyte antigen G (HLA-G) is associated with prognosis in non-small cell lung cancer. Lung Cancer 58, 267-274.

Yie S.M., Yang H., Ye S.R., Li K., Dong D.D. and Lin X.M. (2007c). Expression of HLA-G is associated with prognosis in esophageal squamous cell carcinoma. Am. J. Clin. Pathol. 128, 1002-1009.

Yu P. and Fu Y.X. (2006). Tumor-infiltrating T lymphocytes: friends or foes? Lab. Invest. 86, 231-245.

Zhang L., Conejo-Garcia J.R., Katsaros D., Gimotty P.A., Massobrio M., Regnani, G., Makrigiannakis, A., Gray H., Schlienger K., Liebman, M.N., Rubin S.C., and Coukos G. (2003). Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N. Engl. J.

Med. 348, 203–213.

Zhu C.B., Wang C.X., Zhang X., Zhang J. and Li W. (2010). Serum sHLA-G levels: a useful indicator in distinguishing colorectal cancer from benign colorectal diseases. Int. J. Cancer (Epub ahead of print)

Zou W. (2006). Regulatory T cells, tumour immunity and immunotherapy. Nat. Rev. Immunol. 6, 295-307.