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Deep Insight Section

The HLA-G non classical MHC class I molecule is expressed in cancer with poor prognosis. Implications in tumour escape from immune system and clinical applications

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The HLA-G non classical MHC class I protein has been originally described as being selectively expressed on the invasive trophoblast at fetal-maternal interface at the beginning of pregnancy (Kovats et al., 1990). A few years later, HLA-G protein was detected after fertilization as early as oocyte stage (Jurisicova et al., 1996), and its presence was associated with efficient implantation of fertilized oocyte in uterine mucosa (Fuzzi et al., 2002). Its major contribution to successful pregnancy was also pointed out by both following observations: its reduced expression in pregnancy disorders such as preeclampsia and recurrent spontaneous abortion, was associated with fetal loss (Hviid, 2006) and HLA-G expression by trophoblast was shown to protect fetus from decidual NK cell attack (Rouas-Freiss et al., 1997a). Since then, the expression of HLA-G has been extended to other tissues at immune privileged sites such as: thymus (Crisa et al., 1997), cornea (Le Discorde et al., 2003), pancreas (Cirulli et al., 2006), and the erythroid and endothelial precursors (Menier et al., 2004; Menier et al., 2008). Moreover, its ability to inhibit the effectors functions of decidual NK cells has been demonstrated for allogeneic NK, T, and antigen-presenting cells (APC) (Carosella et al., 2003), which has set HLA-G as a molecule of immune tolerance. In this regard, HLA-G protein was suggested to be a way used to evade the host immune reaction in pathological situations such as infectious diseases (Favier et al., 2007), transplantation (Rouas-Freiss et al., 2007a) and cancer (Rouas-Freiss et al., 2007b).

Tumours employ different strategies to prevent immune responses including tumour-induced impairment of antigen presentation, the activation of negative co-stimulatory signals and the elaboration of immunosuppressive factors. Recently, Schreiber and colleagues (Dunn et al., 2004) propose the cancer immunoediting hypothesis which integrates the different mechanisms of tumour immune escape with the cancer immunosurveillance theory (Burnet, 1957). The cancer immunoediting concept consists of three phases: elimination, equilibrium and escape. The elimination phase corresponds to cancer immunosurveillance and implements cells from innate and adaptative immunity which recognize and destroy tumour cells. In case of partial eradication of tumour cells, equilibrium between the tumour and the immune system develops, that leads to the production of less immunogenic tumour cell clones. Finally, these tumour cell variants escape the antitumour reponse, which results in tumour growth. The expression of the immunotolerant HLA-G protein at tumour site represents one of the immunosuppressive strategies mediated by tumours.

Structural features of HLA-G

In addition to its restricted tissue distribution and its immunotolerant properties, HLA-G has structural particularities. The primary transcript of HLA-G

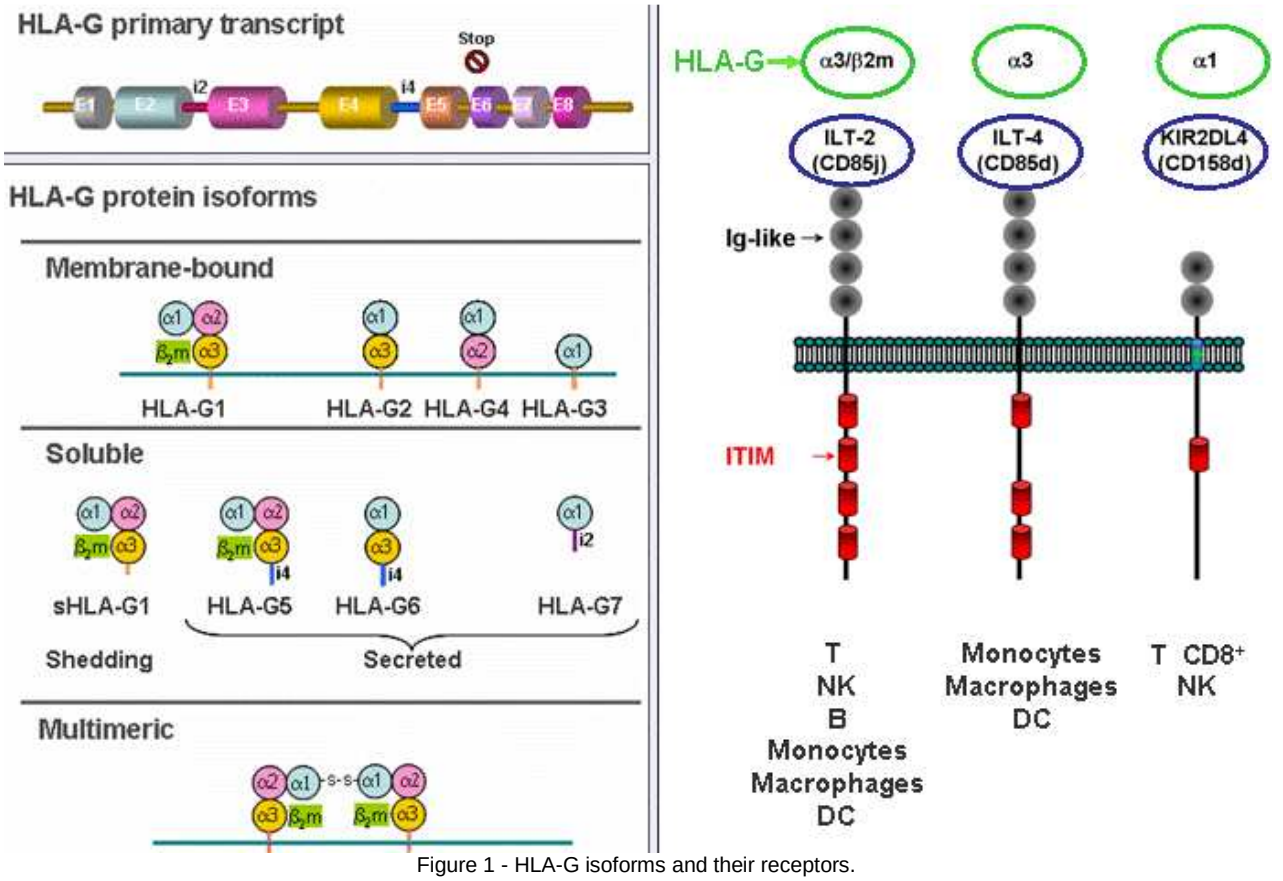

Figure 1 - HLA-G isoforms and their receptors.

gene is alternatively spliced producing seven mRNA encoding four membrane-bound protein isoforms: HLA-G1 to -G4 and three soluble ones: HLA-G5 to -G7 (Ishitani and Geraghty, 1992; Kirszenbaum et al., 1994; Paul et al., 2000a) (Figure 1).

At the structural level, HLA-G1 and its soluble counterpart HLA-G5 are similar to classical HLA class I protein as they include three extracellular domains, the third domain being non covalently associated to ß2-microglobulin. Therefore, among the HLA-G protein isoforms, HLA-G1 and -G5 have been the most studied. Numerous monoclonal antibodies recognizing both isoforms in their properly folded conformation have been developed (Menier et al., 2003), which allowed not only to analyze their pattern of tissue distribution but also to demonstrate the direct role of HLA-G in inhibiting immune responses, by blocking the interactions between HLA-G and its receptors (Khalil-Daher et al., 1999; Le Gal et al., 1999; Riteau et al., 2001c; Selmani et al., 2008). To date, three receptors for HLA-G have been described: one member of the killing immunoglobulin-like receptor (KIR) family: KIR2DL4, which is expressed at NK and CD8+ cell-surface (Cantoni et al., 1998; Rajagopalan et al., 1999), and two members of the immunoglobulin-like transcript (ILT) receptor family : ILT-4 (CD85d) present on myeloid cells (Colonna et al., 1998), and ILT-2, on lymphoid and myeloid cells (Colonna et al., 1997) (Figure 1). While KIR2DL4 is specific for HLA-G (Rajagopalan et al., 1999), ILT-2 and ILT-4 also bind some HLA class I alleles but with a much lower affinity than HLA-G (Shiroishi et al., 2003).

In the past few years, it has been highlighted that HLA-G1 forms dimers at cell-surface of transfected cells but also of trophoblast cells (Boyson et al., 2002; Apps et al., 2007). The HLA-G dimers exhibit higher overall affinity to ILT-2 and -4 receptors than the monomers by significant avidity effects (Gonen-Gross et al., 2003; Gonen-Gross et al., 2005; Shiroishi et al., 2006a; Shiroishi et al., 2006b), suggesting that the active conformation of HLA-G is the dimeric form (Apps et al., 2007; Gonen-Gross et al., 2003). In addition, the association of HLA-G heavy chain with ß2m is required for interaction with ILT-2, but not for binding to ILT-4 (Gonen-Gross et al., 2005; Shiroishi et al., 2006). Recently, soluble HLA-G1 (shed HLA-G1) and HLA-G5 proteins were detected in body fluids such as plasma from hepato-renal transplanted patients (Shiroishi et al., 2006b) and malignant effusions (Creput et al., 2003; Davidson et al., 2005), through the development of enzyme-linked immunosorbent assays (ELISA) of which two HLA-G-specific ones were validated during an international workshop (Rebmann et al., 2005; Rebmann et al., 2007).

The other HLA-G isoforms differs from HLA-G1 and -G5 by the lack of one (HLA-G2, -G4, and-G6) or two (HLA-G3 and -G7) extracellular domains

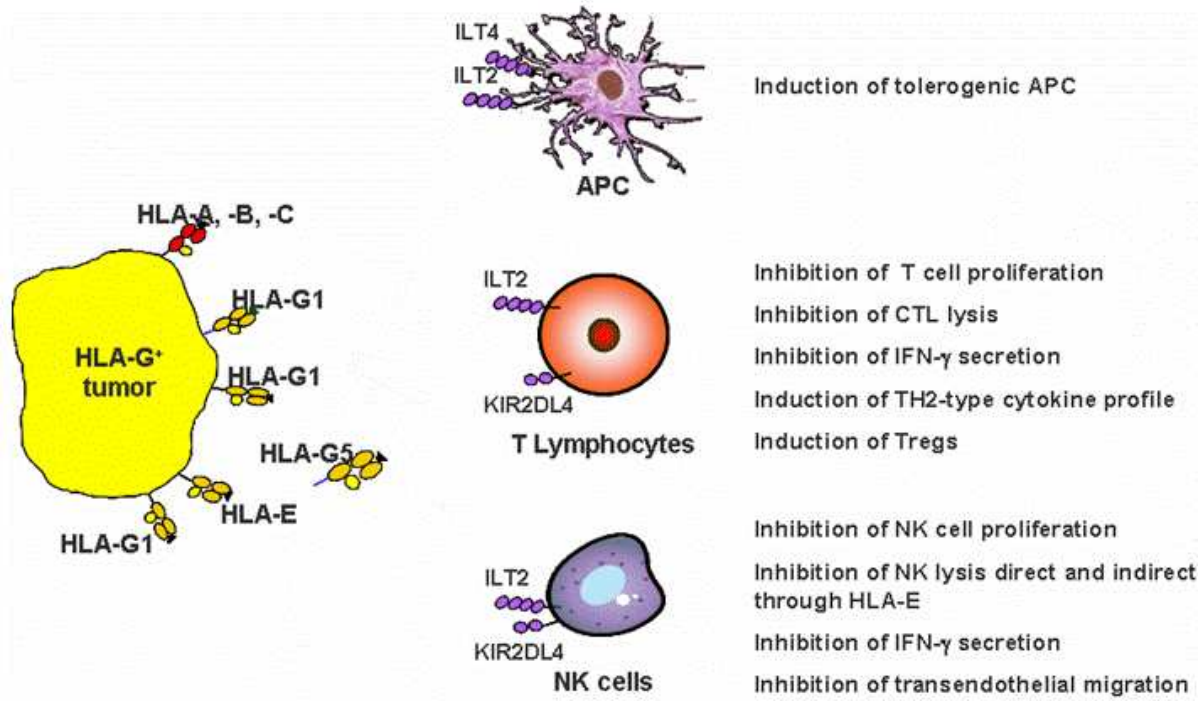

Figure 2 - HLA-G properties towards immune cells.

(Carosella et al., 2003). Their conformational structure remains to be determined. Although their detection is still difficult, the availability of an antibody directed against a peptide in the a1 domain common to all HLA-G isoforms allowed their characterization (McMaster et al., 1998; Paul et al., 2000b; Menier et al., 2000; Lozano et al., 2002). Cell-surface expression of these truncated isoforms is probably dependent on the type of cell in which they are expressed (Mallet et al., 2000; Bainbridge et al., 2000a; Riteau et al., 2001a; Riteau et al., 2001b). Their detection as membranebound proteins was related to the same ability as the full-length HLA-G isoform to inhibit NK and antigen-specific cytotoxic T cell responses (Riteau et al., 2001b).

Functions of HLA-G protein isoforms

Through interaction with the above-described receptors, HLA-G has been shown to inhibit all the actors of the anti-tumour response (Figure 2).

Membrane-bound HLA-G reduces NK cell-mediated cytolysis, whether HLA-G is the only inhibitory ligand present on the surface of target cells (Khalil-Daher et al., 1999; Rouas-Freiss et al., 1997b), or is co-expressed with other inhibitory ligands including classical HLA class I antigens and the non classical HLA-E protein (Rouas-Freiss et al., 1997a; Riteau et al., 2001c) and/or activating ligands like the MHC class I-related chain-A (MICA) (Riteau et al., 2001b; Menier et al., 2002). HLAG also protects these target cells from antigen-specific cytotoxic T lymphocyte (CTL) activity either directly by interaction with the above-mentioned inhibitory receptors (Le Gal et al., 1999; Riteau et al., 2001b), or indirectly, by inhibiting the proliferative response of CD4+ T lymphocytes (Riteau et al., 1999), which thus leads to the decrease of the cooperation between CD4+ with CD8+ T cells. HLA-G1 is also able to exert a direct suppressive effect on CD4+ T cells (Bainbridge et al., 2000b). Furthermore, HLA-G1-expressing antigen presenting cells (APC) render CD4+ T cells anergic and the pre-sensitization of CD4+ T cells by HLA-G1+APC confers them immunosuppressive properties (LeMaoult et al., 2004; Naji et al., 2007). Recently, another mechanism inducing suppressor T or NK cells has been highlighted. These properties are acquired temporary through the rapid transfer of membrane patches (termed trogocytosis) containing HLA-G, from APC or tumour cells to T or NK cells (LeMaoult et al., 2007; Caumartin et al., 2007). Finally, cytokinemediated effects represent a means by which HLA-G can exert immunosuppression. In this regard, HLA-G influences the balance of Th(T helper)1/Th2 cytokines secretion by rather promoting Th2 type responses (Maejima et al., 1997; Kanai et al., 2001).

Like their membrane-bound counterparts, soluble HLA-G proteins (sHLA-G) have immunosuppressive properties through similar mechanisms, but with distinct characteristics. Soluble HLA-G antigens have been shown to inhibit NK cell-mediated cytotoxicity (Selmani et al., 2008; Park et al., 2004; Marchal-Bras-Goncalves et al., 2001; Poehlmann et al., 2006). Recently, following interactions between sHLA-G and the KIR2DL4 receptor on resting NK cell-surface, NK cells were shown to be activated and to release a set of chemokines and cytokines driving a proinflammatory / proangiogenic response (Rajagopalan et al., 2001). An

opposite effect on angiogenesis has been observed by inducing endothelial cell apoptosis upon their binding to the CD160 (By55) receptor (Fons et al., 2006). Like soluble HLA class I antigens, through upregulation of FasL following the interactions of sHLA-G with CD8, activated T lymphocytes and NK cells come into apoptosis (Fournel et al., 2000; Contini et al., 2003). Moreover, sHLA-G inhibits the cytotoxic activity of antigen-specific CTL (Contini et al., 2003). They also decrease CD4+ and CD8+ T cell alloproliferation (Selmani et al., 2008; Contini et al., 2003; Lila et al., 2001; Le Friec et al., 2003), by blocking cell cycle progression (Bahri et al., 2006). Like its membrane-bound counterpart, naive T cells pre-sensitized by HLA-G5 differentiate into suppressor T cells (Le Rond et al., 2006). These suppressor T cells are not conventional regulatory T cells. Indeed, they express lower CD4 and CD8 antigens, which belong to the TcR/CD3 complex. Such down-modulated co-receptors T cells are hyporesponsive to allogeneic stimulus (Naji et al., 2007). Lastly, a particular property of sHLA-G is their ability to induce tolerogeneic dendritic cells associated with inhibition of their differentiation (Ristich et al., 2005). Of note, we showed that HLA-G-expressing melanoma cell lines could release exosomes which bear HLA-G together with well-described proteins as Lamp-2 (Riteau et al., 2003). The secretion of exosomes was shown to be another way for tumour to suppress immune responses (Valenti et al., 2007). Whether these HLA-G+ exosomes are immunosuppressive remains to be determined.

HLA-G expression in tumour lesions and malignant effusions

Given that HLA-G is expressed on trophoblast which is defined as a pseudo-malignant tissue, our group was the first to analyze the presence of this protein on malignant lesions. Melanoma was chosen for this study because MAGE antigens and melanoma cell adhesion molecules (Mel-CAM) are expressed in both melanoma and trophoblast cells. Moreover, this tumour was the most studied from an immunological point of view. Thus, in 1998, we described a high level of HLA-G in a skin biopsy from melanoma metastasis and in a melanoma cell line for which presence of HLA-G was related to protection from NK lysis (Paul et al., 1998). Two following studies on two hundred patients melanoma biopsies revealed that: (i) HLA-G protein was expressed in thirty percent of the patients (Paul et al., 1999; Ibrahim el et al., 2004), (ii) HLA-G expression is associated with malignant transformation of melanocyte as this protein was detected in both primary and metastatic tumour sites, but neither in adjacent tumour tissue or in spontaneous tumour regression site or in healthy skin (Paul et al., 1999; Ibrahim el et al., 2004), (iii) higher levels of inflammatory tumour-infiltrating cells were observed in malignant melanoma lesions in comparison to begnin ones (Ibrahim el et al., 2004) and (iv) up-regulation of HLA-G in melanocytes is a better predictor of malignancy than classical HLA class I antigens defects, (Ibrahim el et al., 2004) which are often described as an important mechanism of tumour escape from immunosurveillance (Ferrone and Marincola, 1995). This association between the presence of HLA-G and the malignant nature of the tumour suggested that HLA-G was a mechanism for tumours to escape immune surveillance.

Following our first description, HLA-G expression in melanoma lesions was confirmed by other groups and numerous other tumour lesion types of either ectodermic or mesodermic or endodermic origin were studied. Today, in about two thousand patients analyzed, HLA-G protein was found in almost all types of cancer whatever their origin, but in varying proportions ranging between 10% (leukemia) to 95% (esophageal squamous cell carcinoma) (Figure 3).

A recent review summarizes HLA-G protein expression in tumour lesions (Carosella et al., 2008). Since that review, among 140 patients with lung cancer analyzed, approximately 60% of them expressed HLA-G protein. Two other types of cancers were studied: in 175 patients with classical Hodgkin lymphoma, HLA-G protein was found in 50% of cases and in 121 patients with esophageal squamous cell carcinoma, this proportion reached 95%. This heterogeneity may reflect the differences in the biology of individual tumours, in study design and/or the sensitivity of the methods used to detect HLA-G protein in malignant lesions. HLA-G expression is also heterogeneous within a tumour type and within individual lesions. Indeed, HLA-G has been detected in the tumour tissue and/or in the infiltrating lymphocytes in tumours such as melanoma (Ibrahim et al., 2003), breast cancer (Lefebvre et al., 2002), and lung carcinoma (Urosevic et al., 2001). Moreover, HLA-G expression can concern different number of cells within a type of tumour according to the patients (Ibrahim el et al., 2004; Nuckel et al., 2005).

Through the recent development of HLA-G-specific ELISA, high levels of HLA-G in its soluble form have also been detected in the plasma of patients with various cancers (Rebmann et al., 2003), including melanoma (Ugurel et al., 2001), glioma (Wiendl et al., 2002), multiple myeloma (Leleu et al., 2005), lymphoblastic and monocytic acute leukaemia (Amiot et al., 2003; Gros et al., 2006), neuroblastoma (Morandi et al., 2007), and in ascites from breast and ovarian carcinomas (Singer et al., 2003).

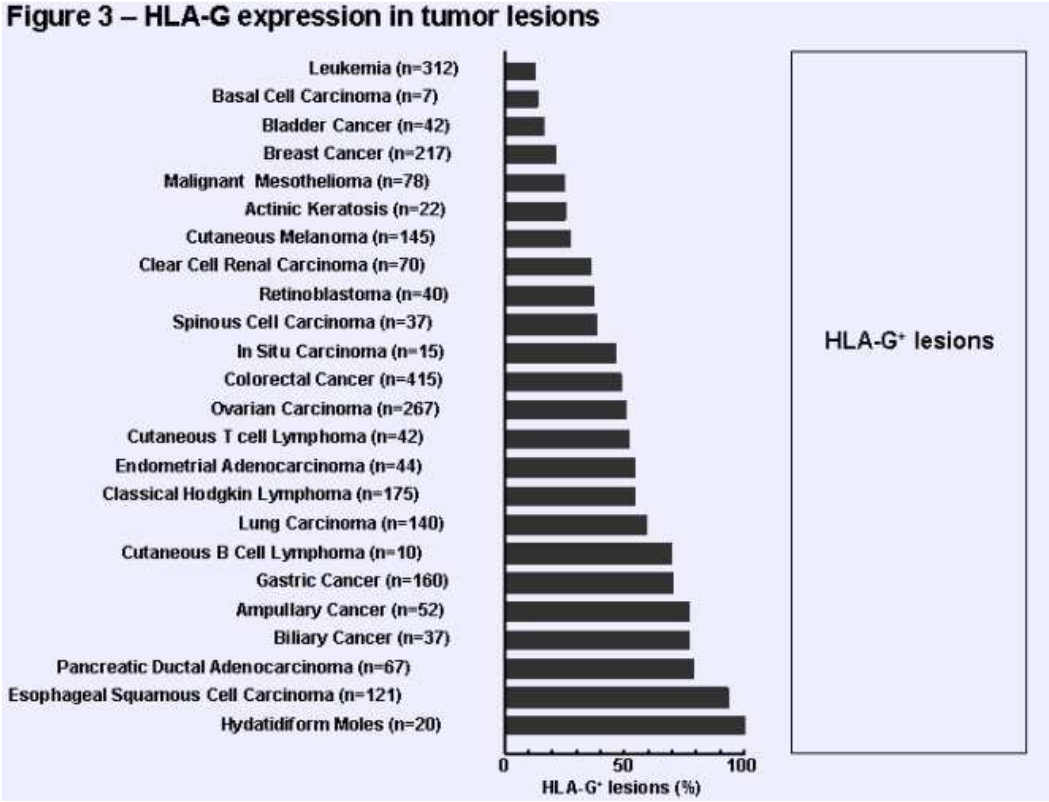

Figure 3 - HLA-G expression in tumor lesions.

Regulation of HLA-G expression in cancer

Up to date, in more than two hundred cell lines derived from malignant tumour biopsies, HLA-G protein was only detected in about ten ones, what contrasts with the proportion of surgically removed malignant tumour lesions which express HLA-G (Rouas-Freiss et al., 2003). This discrepancy shows that in vitro, factors which were maintaining the expression of HLA-G are not present anymore, and that in vivo, HLA-G expression is activated by environmental stimuli such as stress conditions, cytokines and epigenetic variations.

Indeed, like MICA genes, we showed that HLA-G is a stress-inducible gene. Heat shock and arsenite induced an increase of the different HLA-G alternative transcripts without affecting the other MHC class I HLA-A, -B, -C, -E and -F transcripts in melanoma cells (Ibrahim et al., 2000). A stress situation is represented by hypoxia, which exists in the surrounding microenvironment of rapidly growing tumours. Hypoxia was shown to induce the hypoxia-inducible factor-1a (HIF-1a) which can in turn, trigger the transcription and traduction of HLA-G gene in HLA G- tumour cells (Chang and Ferrone, 2003; Mouillot et al., 2007; Gazit et al., 2007). In contrast, HLA-G expression was decreased in HLA-G+ tumour cells (Mouillot et al., 2007). In both up- and down-modulation, HLA-G expression depends on HIF-1a stabilization (Mouillot et al., 2007). Finally, it is well-known that both the depletion of the essential amino acid tryptophan and the accumulation of tryptophan metabolites from the microenvironment provokes an inhibition of immune cells function. This represents another tumour escape mechanism from immune system as some tumours or APC in tumour-draining lymph nodes express the indolamine 2,3-dioxygenase (IDO), an enzyme which metabolizes tryptophan (Friberg et al., 2002; Uyttenhove et al., 2003; Munn and Mellor, 2007; Munn et al., 2004). Searching for a link between HLA-G and IDO molecules, we found that (i) inhibiting the function of IDO up-regulates HLA-G1 cell-surface expression on APC and tumour cell lines (Gonzalez-Hernandez et al., 2005), (ii) IDO induces HLA-G expression during monocyte differentiation into dendritic cells (Lopez et al., 2006), and (iii) IDO and HLA-G inhibit T cell alloproliferation through two independent but complementary pathways (Le Rond et al., 2005). Thus, the recent development of IDO inhibitors as a new immunoregulatory treatment modality for clinical trials (Katz et al., 2008) has to consider the possible stimulation of HLA-G expression.

Numerous studies have investigated the cytokine-mediated induction of HLA-G expression. Particularly, the anti-inflammatory and immunosuppressive IL-10 cytokine secretion by lung carcinoma cells and T and B lymphoma cells has been correlated with concomitant HLA-G expression (Urosevic et al., 2001; Urosevic et al., 2002). Transactivation of HLA-G transcription has also been demonstrated by JEG3 choriocarcinoma cell exposure to leukemia inhibitory factor (LIF) (Bamberger et al., 2000). Furthermore, interferon

(IFN)-a, -ß and -γ enhance HLA-G cell-surface expression by tumours or monocytes (Ugurel et al., 2001; Lefebvre et al., 1999; Lefebvre et al., 2001; Wagner et al., 2000; Moreau et al., 1999; Bukur et al., 2003). This up-regulation of HLA-G at the tumour site represents one potential side effect of the administration of IFN for immunotherapy and may confer immunoprotection to tumour cells, thus favouring tumour expansion. In this regard, an association has been established between the lack of clinical response to therapy with IFN-a high doses and HLAG expression in melanoma lesions (Wagner et al., 2000). The expression of inflammatory cytokines such as IFN-γ is under the control of the nuclear factor-κB (NF-κB), a pivotal transcription factor of innate and adaptative immunity. In addition, NF-κB signaling also plays a critical role in cancer development and progression (Karin and Greten, 2005). Thus, we analyzed whether NF-κB and HLA-G were linked and demonstrated that NF-κB activation enhances HLA-G intracytoplasmic tumour cell content, but promotes the proteolytic shedding of membrane-bound HLA-G (Zidi et al., 2006). Moreover, we showed that HLA-G1+ tumour cells activate NF-κB in NK cells. This activation occurs through interactions between the a1 domain of HLA-G and presumably the KIR2DL4 receptor (Guillard et al., 2008).

Remarkably, cytokines have no effect on HLA-G gene transcription in tumour cells in which this gene is repressed (Frumento et al., 2000). This led us to propose the hypothesis of the existence of epigenetic mechanisms, which may activate the HLA-G gene in some tumours. Global genomic hypomethylation occurs frequently during carcinogenesis and genetic lesions in methyl-chromatin-related genes, such as histone deacetylase, are supposed to influence the epigenetic alterations involved in cancer (Esteller et al., 2002; Esteller and Herman, 2002). We showed that exposure of some tumours cell lines to histone deacetylase inhibitors, or the decitabine DNA demethylating agent, reactivate both HLA-G gene transcription and traduction (Moreau et al., 2003; Mouillot et al., 2005; Yan et al., 2005). Thus, the HLA-G gene derepression must be considered as adverse effect following chemotherapy with drugs such as decitabine, which are currently used to reactivate tumour suppressor genes and other genes involved in invasion and metastasis (Maio et al., 2003).

HLA-G in cancer immunoediting

During the elimination phase which matches with cancer immunosurveillance, classical HLA class I expression at tumour cell-surface is supposed to be unchanged. Tumour-infiltrating lymphocytes and NK cells produce Th1-type cytokines. Particularly, IFN-gamma is one of the cytokines up-regulating HLA-G expression in tumour cells either directly or indirectly through induction of IDO. Towards its predominant inhibitory role, HLA-G could greatly weaken host anti-tumoural immune responses.

During the equilibrium phase which corresponds to cancer persistence, epigenetic changes take place frequently and contribute to the development of non immunogenic tumour cell clones. At present, in vitro studies showed that demethylation and histone deacetylation reverses HLA-G gene silencing. In addition to its direct inhibitory role on immune cells, HLA-G protein may also play this role indirectly through the plasma membrane stabilization of the non classical CMH class I HLA-E protein (Khalil-Daher et al., 1999; Riteau et al., 2001b; Borrego et al., 1998). HLA-E reaches cell surface through binding of MHC class I leader peptide (Braud et al., 1997; Braud et al., 1998; Lee et al., 1998). Although classical HLA class I molecules can be completely lost, HLA-G can mediate the membrane expression of HLA-E, which confers additional protection of tumour cells to NK cytolysis. Moreover, HLA-G also contributes to the already altered antigen presentation (Wright and Ting, 2006) by down-modulating HLA class II molecules on APC (Ristich et al., 2005).

The escape phase in which cancer progresses, is the phase where HLA-G is preferentially expressed comparatively to initial malignant tumour lesions. Tumours generate an appropriate microenvironment that allows them to prevent their immune cell elimination, thus favouring their growth. The mechanisms to achieve this goal include the modulation of antigen expression that allows preventing activation of the immune system, the induction of peripheral tolerance by induction of anergy or induction of immunosuppressor cells, and the production of immunosuppressive cytokines. HLA-G plays a significant role in these mechanisms because HLA-G remains the almost single-molecule expressed by tumours. In addition to local effects at its site of expression, secreted soluble HLA-G could also have systemic inhibitory activity through its distribution in blood circulation. Among the immunosuppressive cytokines produced by tumours, IL-10 is responsible for HLA-G up-regulation in cancer. Both IL-10 and HLA-G may be produced by tumour cells themselves or by tumour-infiltrating cells. There is an amplification loop since both IL-10-induced decrease of the production of Th1-type cytokine and HLA-G expression are able to increase IL-10 production. Moreover, HLA-G was shown to increase its own inhibitory receptors in NK, APC and T effector cells (LeMaoult et al., 2005), and IL-10 can also modulate the KIR repertoire on NK and T cells, what further contribute to dampen immune responses. During this phase, chronic inflammation is assumed to favour tumour growth through activation of NF-κB, which may enhance systemic inhibitory action through the release of soluble HLA-G1 from proteolytic shedding of membrane HLA-G1. The rapid tumour cell proliferation creates a hypoxic microenvironment, a stress condition which also promotes tumour invasion per se but in addition, by inducing HLA-G expression.

Importantly, some therapeutic strategies, as either immunotherapy using IFN-a, or chemotherapy with DNA-demethylating or histone deacetylating agents, or therapeutic vaccination using IDO inhibitors, must be revisited since these treatments were shown to upregulate HLA-G.

Biological relevance of HLA-G expression in cancer

In vitro studies have shown that HLA-G-endogenously expressing melanoma, glioma and renal carcinoma cell lines are protected from lysis by alloreactive NK and lymphokineactivated killer cells and/or antigen-specific CD8+ T cells (Paul et al., 1998; Wiendl et al., 2002; Bukur et al., 2003). This protective effect was directly due to HLA-G expression by tumour cells since the blockade of this molecule restored the cytotoxic activity of effector cells. Since then, two studies have reinforced the role of HLA-G in tumours. In the first study, we derived a HLA-G+ melanoma cell line, called Fon, from a HLA-G+ melanoma biopsy (Rouas-Freiss et al., 2005). The Fon cell line expressed high levels of membrane-bound HLA-G1, which confers resistance to NK cell line lysis through interaction with ILT-2 inhibitory receptor. During the long-term spread of Fon cells in culture, the expression of HLA-G1 has been lost, as was its protection against the NK cell cytotoxicity. Although IFN-ß, -γ or decitabine treatments enhanced HLAG1 expression in the primary Fon cells, neither these cytokines, nor this DNA-demethylating drug brought back HLA-G1 transcription. Altogether, these results emphasize the difficulty using cell lines derived from tumour biopsies to establish the physiopathological relevance of HLA-G in anti-tumour responses since HLA-G expression can be lost during long-term tumour expansion. Moreover, they support the role of HLA-G1 expressed at tumour cellsurface in preventing host innate immune cell responses.

As described for other tumour type, the second study showed that neuroblastoma patients had significant higher serum levels of sHLA-G than healthy donors. The source of sHLA-G in neuroblastoma patients was not tumour cells but monocytes in an activated state. The sHLA-G produced was able to inhibit CTL and NK cell-mediated cytotoxicity against tumour cells. Interestingly, the sHLA-G-secreting monocytes were instructed by neuroblastoma cells through the release of soluble factors that may be IL-10 or TGF-ß1. These monocytes display features of macrophage-like activated cells but shift towards a more anergic phenotype since they secrete higher levels of immunosuppressive sHLA-G and lower IL-12, a cytokine which promotes anti-tumour responses. These findings support an in vivo systemic effect of HLA-G in cancer (Morandi et al., 2007), and provide guidance on a novel mechanism of tumour immune evasion.

Clinical significance of HLA-G expression in cancer

As above described, HLA-G expression has always been detected in malignant tissues or effusions and has never been found neither in healthy tumour surrounded areas, nor in tissues or effusions from patients suffering of begnin disease, nor in the corresponding tissues from healthy individuals (Ibrahim el et al., 2004; Ibrahim et al., 2003; Singer et al., 2003; Aractingi et al., 2003). In melanoma, upregulation of HLA-G molecules in melanocytic cells appears as a better predictor of malignancy than classical HLA class I antigen defects frequently observed in this cancer type (Ibrahim el et al., 2004).

The idea that HLA-G expression could be a prognostic factor has emerged recently. Indeed, HLA-G expression and/or sHLA-G high levels have been significantly correlated with poor prognosis in non-small cell lung cancer, melanoma, glioblastoma, ovarian carcinoma, B-CLL, cutaneous T cell lymphoma, neuroblastoma and digestive cancers (Urosevic et al., 2001; Nuckel et al., 2005; Ugurel et al., 2001; Wiendl et al., 2002; Morandi et al., 2007; Singer et al., 2003; Urosevic et al., 2002; Yie et al., 2007a; Yie et al., 2007b; Ye et al., 2007; Yie et al., 2007c). In particular, in ovarian carcinomas, high levels of soluble HLA-G protein were measured in the effusions produced in late-stage disease which overlaps with the first appearance of metastases (Singer et al., 2003). In digestive cancers and B-CLL, HLA-G expression was shown as being an independent prognostic factor (Nuckel et al., 2005; Yie et al., 2007b; Ye et al., 2007; Yie et al., 2007c). In multivariate analysis of B-CLL patients, HLA-G expression was an even better independent prognostic factor than the zeta-associated protein 70 (ZAP-70) or CD38 status (Nuckel et al., 2005). Finally, the role of HLA-G in tumour escape from host immune cell is emphasized by its involvement of the resistance to IFN therapy observed in some melanoma patients (Wagner et al., 2000).

Conclusion and perspectives

Towards its immunotolerant properties, HLA-G expression is an additional but efficient way for tumour to evade immunosurveillance. In the few last years, the clinical relevance of HLAG has been established in cancer. Indeed, HLA-G was always correlated with higher grade histology and advanced disease stage, and for the most complete studies, increased depth of invasion, more frequent lymph node metastasis, reduced host immune response and shortened survival. However, multicenter studies including larger cohorts of patients are needed to demonstrate the usefulness of HLA-G levels quantification in predicting the clinical outcome of cancer patients. Since clinical studies clearly reveal a negative correlation between HLA-G

and patient survival, HLA-G appears an attractive molecular target to develop new anti-tumour therapies. The reverse of tumour growth might be obtained through blockade of HLA-G synthesis by preventing its transcription using silencing RNA or acting on HLA-G alternative splicing (Rouas-Freiss et al., 2005), or through restoration of host immune responses by using antibodies neutralizing interactions between HLA-G and its receptors. Thus, the in vivo validation of the proof-of-concept requires the development of a tumour model in mice since although a true HLA-G homolog is lacking, murine PIR-B can bind HLA-G allowing such model to be evaluated there is a HLAG inhibitory receptor homolog (Liang et al., 2002; Ungchusri et al., 2001; Comiskey et al., 2003; Chiang et al., 2002; Chiang and Stroynowski, 2005).

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