HLA-G in Skin Cancer: A Wolf in Sheep’s Clothing?

Mirjana Urosevic and Reinhard Dummer

ABSTRACT: Despite well-defined and immunogenic tumor antigens, and even in the presence of tumor antigen-specific cytotoxic cells, the immune system does not appear to be very effective in eradicating cells that have undergone malignant transformation. Tumor cells, even though invading and representing a threat, are not truly “foreign” but autologous cells that have become transformed in a subtle way, enabling them to escape the host immune system. Melanoma, and to less extent nonmelanoma, skin cancers have developed different strategies to circumvent host immunosurveillance. HLA-G is one of the molecules implicated in cancer immunescape. This review will concentrate on induction and expression of this nonclassical class I molecule in different skin cancer types presenting existing experimental evidence on this topic. Human Immunology 64, 1073–1080 (2003). © American Society for Histocompatibility and Immunogenetics, 2003. Published by Elsevier Inc.

KEYWORDS: HLA-G; epithelial skin cancer; melanoma; immunescape

INTRODUCTION

The immune system in continuously patrolling the body to detect and destroy “foreign, potentially invading agents” that may be detrimental to our health. It is a balancing system of “activation and inhibition” that leads to elimination of the intruder while avoiding illicit, self-destructive reactions. Apart from infectious pathogens, our immune system seems capable of recognizing and destroying nascent transformed cells, a concept known as cancer immunosurveillance [1]. However, despite well-defined and immunogenic tumor antigens, and even in the presence of tumor antigen-specific cytotoxic cells, the immune system does not appear to be very effective in eradicating cells that have undergone malignant transformation. Tumor cells, even though invading and representing a threat, are not truly “foreign” but autologous cells that have become transformed in a subtle way enabling them to escape host immune system in different ways. Human leukocyte antigen G (HLA-G) is one of the molecules implicated in cancer immunescape. Under physiologic conditions, expression of HLA-G is confined to immunoprivileged sites, such as placenta [2–4]. There it is presumed to be responsible for the functional silencing of the immune response leading to the tolerance of semiallogeneic fetus during the pregnancy. HLA-G seems to affect almost every aspect of human immunity. In vitro, HLA-G was found to inhibit allogeneic T-cell and natural killer (NK) cell cytotoxicity as well as T-cell proliferative response [2–4]. Given the fact that the placenta lacks expression of HLA class I (HLA-A, -B) and all class II genes, a situation often encountered in cancer, the expression of HLA-G is thought to provide tumor cells with an effective mechanism to counteract the host immune system [5, 6]. To date, ectopic expression of HLA-G was reported in different types of human cancer, strongly implying that HLA-G may indeed have a role in tumor immunology in vivo [2, 5, 7].

The importance of the immune system in controlling cancer growth is particularly reflected in the fact that immunosuppressed individuals have significantly higher risk of developing cancer as compared to general population [1]. Cancers of the skin appear to be the best examples for this observation. In transplant recipients, squamous cell carcinoma of the skin is occurring up to 250 times as frequently as in the general population [8]. Furthermore, up to fourfold increase in the incidence of de novo malignant melanoma after organ transplantation has been reported [1, 8]. Skin, in contrast to other organs, is continuously exposed to ultraviolet radiation (UVR), which is one of the most important risk factors for the development of skin cancer in general [8].
addition to induction of mutations in p53 tumor-suppressor gene [9, 10], the exposure to UVR leads to the local immune suppression [11]. UVR depletes Langerhans’ cells (LCs) in the skin and negatively affects their antigen-presenting function [11]. UVR also stimulates keratinocytes to produce isomerized urocanic acid, tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10), all of which promote the development of suppressor T cells [11]. TNF-α and urocanic acid also serve to deplete LCs from the epidermis, as do many immunosuppressive drugs. Tumors, such as melanoma and to less extent nonmelanoma skin cancers (NMSCs), have developed additional strategies enabling them to circumvent host immunosurveillance. Structural and functional alteration of antigen-presenting (HLA) molecules, loss of tumor antigen expression, lack of costimulatory molecules, and production of immunosuppressive cytokines are some of the mechanisms employed by the tumors to escape immune recognition [5, 12]. Given the beneficial role of HLA-G in establishing immune tolerance, HLA-G expression has been initially investigated in melanoma and recently in NMSC as a factor permitting immune escape. This review will concentrate on induction and expression of this nonclassical class I molecule in different skin cancer types presenting existing experimental evidence and trying to delineate putative functions of HLA-G in human cancer.

MELANOMA

Partial or complete losses of HLA class I molecules have often been reported in melanoma [12–15]. According to the “missing self-hypothesis” [16], melanoma cells that have lost HLA class I antigen expression should have become directly susceptible to NK cell lysis. Despite the findings that melanoma lesions are frequently infiltrated with NK cells [17], HLA class I loss melanoma cell variants continue to grow and are not being destroyed by surrounding NK cells. This immunologic conundrum led some to the assumption that other mechanisms (molecules) might be involved in evasion of the host immune response. Based on its mainly immunoinhibitory functions, HLA-G appeared to be a suitable candidate for this role. In 1998, Paul et al. [18] provided first evidence that functional HLA-G protein expression protects melanoma cell lines from NK-mediated cell lysis. Shortly thereafter, the same workgroup analyzed HLA-G expression in a larger panel of ex vivo tissue specimens [19]. In primary and metastatic melanoma lesions, reverse-transcriptase–polymerase chain reaction (RT-PCR) and Western blotting demonstrated heterogeneous HLA-G expression on transcriptional and protein level, respectively. HLA-G message was more abundant in tumor samples as compared to healthy skin. The authors could demonstrate that in one patient HLA-G protein expression colocalized with HLA class I and gp100 (HMB45) expression in primary skin tumor and lymph node metastasis, but not in the healthy skin and a tumor regression site. Our study analyzing expression of classical (HLA-A, -B) and nonclassical (HLA-G) class I molecules by real-time quantitative PCR in primary melanoma cell cultures demonstrated heterogeneous HLA-G expression that was in orders of magnitude lower that the expression of classical HLA class I molecules [20]. HLA-G expression on the transcriptional level did not inversely correlate with the reduced expression of HLA-A and -B, suggesting that HLA-G expression represents an independent characteristic of individual melanoma metastasis [20].

In 1999 Real et al. [21] published results of a broad screening of a variety of neoplastic cell lines and tissues, including five melanoma cell lines and nine tissue specimens, that exhibited no constitutive HLA-G protein expression in tested samples either by flow cytometry or immunohistochemistry. Nevertheless, the authors demonstrated rather ubiquitous expression of HLA-G on mRNA level in tissues as well as in cell lines. In addition, the authors failed to reveal the potential inducibility of HLA-G expression by interferon-γ (IFN-γ) in melanoma cell lines. This report caused a real stir in the field whether HLA-G plays any role at all in melanoma immune escape. These controversial findings were tentatively explained by a strong post-transcriptional regulation of HLA-G expression in melanoma cells that is responsible for the absence of protein expression despite detectable, but low, mRNA levels. This report was followed by additional two reports by Polakova and Russ [22] and Frumento et al. [23], which failed to demonstrate any constitutional (as well as IFN-γ inducible [23]) expression in almost all melanoma cell lines that they have investigated by flow cytometry. It is conceivable that described discrepancies in pattern and presence of HLA-G expression in melanoma are merely reflecting the tumor (cell line) heterogeneity and the complexity of HLA-G regulatory mechanisms rather then the differences in methodology used in these studies.

Another aspect of HLA-G expression in melanoma has been addressed by a notion that HLA-G expression can be induced by interferons [24], even though this concept has been disproved by some of the studies mentioned above. Melanoma patients with advanced disease are often treated with interferon-α that has been found to prolong relapse-free and overall survival in this group of patients [25, 26]. It would therefore seem imaginable that in these patients interferon-α treatment through HLA-G upregulation would additionally increase the chances of melanoma cells’ immune escape. Wagner et al. [27] have analyzed the expression pattern of classical as well as nonclassical HLA class I molecules in melanoma
tissue specimens in order to evaluate their potential impact on clinical response to interferon-α treatment. By doing retrospective analysis of tissue samples obtained prior to interferon-α treatment, the authors indicate that tumor lesions of patients that presented with progressive disease displayed total loss of classical HLA class I expression together with moderate HLA-G up-regulation, whereas relapse-free patients demonstrated tumor lesions that had unaltered expression of classical HLA class I molecules with or without concomitant HLA-G expression. These results suggest that in addition to loss of classical HLA class I antigens, ectopic HLA-G expression might be underlying melanoma unresponsiveness to immunomodulatory treatment with subsequent immune-scape. A recent study by Ugurel et al. [28] reported elevated serum levels of soluble HLA-G (sHLA-G) in melanoma patients that correlated well with the advanced disease stage and tumor load. Serum sHLA-G levels had no impact on the course of the disease and patient’s survival. Patients that underwent immunomodulatory treatment with interferon-α exhibited an increase in serum sHLA-G levels. Besides, the authors could demonstrate that IFN-α is capable of upregulating HLA-G cell surface expression on peripheral blood monocytes of melanoma patients. The authors conclude that elevated concentrations of sHLA-G in melanoma patients’ sera might not be originating from melanoma cells themselves, but more likely by shedding or secretion from circulating monocytes. The significance and exact impact of soluble HLA molecules on immune response in cancer patients remains yet to be elucidated.

Apart from putative upregulation of HLA-G, another mechanism might confer melanoma cells privilege of immune evasion. NK cells express activating receptors, such as NKG2D, which bind to stress-induced ligands (MICA and MICB) than can be upregulated in cancer, including melanoma [29]. Activation of NK cells through these molecules can overcome the inhibitory effect of HLA class I binding receptors (KIRs) [30, 31]. In their recent report, Menier et al. [32] have revealed that HLA-G expression may represent a powerful way to switch off NK cells activated by MICA coexpressed on the same tumor cells. In this manner, HLA-G expression in the tumor expressing MICA(B) might enable tumor cells to counteract an immune attack of infiltrating cells against a MIC-positive tumor.

Tumor-cell targeted immune response can also be modulated by presence or absence of cytokines in tumor immediate microenvironment. Tumor cells produce different cytokines and chemokines that can negatively affect maturation and function of immune cells. IL-10 is one of such cytokines, whose overexpression has been reported in melanoma [33–36]. IL-10 exerts a variety of biologic effects creating immunosuppressive microenvironment in favor of the tumor. IL-10 is one of the cytokines implicated in the induction of HLA-G [37–40]. Through upregulation of HLA-G, IL-10 might be additionally abrogating antitumor immune response in melanoma [5]. Studies to come will provide more insight into this topic.

NONMELANOMA SKIN CANCER

Cutaneous Lymphomas

Primary cutaneous lymphomas comprise a spectrum of heterogeneous entities characterized by clonal accumulation of lymphocytes initially restricted to the skin [41]. Immune abnormalities, such as decreased cell-mediated cytotoxicity, decreased T-cell response to antigens, and several other immune phenomena, have been reported in cutaneous lymphoma patients [42, 43]. Most of these abnormalities may be attributed to pleiotropic immunosuppressive effects of IL-10 whose expression has been demonstrated in different cutaneous lymphoma types [44–46]. Despite the evidence of antitumor humoral and cellular immune response [47–50], cutaneous lymphoma may and can progress eventually killing the patient. Knowing that loss of HLA class I molecules represents a rare event in this group of diseases [51, 52], it is less likely that this mechanism would account for the stealthness of the malignant clone. Both B and T lymphocytes, on the other hand, are transcribing and capable of expressing HLA-G under different culture conditions [53–55]. Under the assumption that overexpression of IL-10 in cutaneous lymphomas might lead to the upregulation of HLA-G, we assessed HLA-G and IL-10 expression in a panel of 45 cutaneous lymphoma patients [56]. Immunohistochemistry revealed HLA-G protein expression in 23 patients (51%). HLA-G protein was mostly expressed in indolent B-cell lymphomas as well as in T-cell lymphoma patients of advanced disease stage and high-grade histology. We could demonstrate coexpression of IL-10 in 16 (73%) of 22 HLA-G positive patients (p < 0.001). Interestingly, the shift from Th1 to Th2 cytokine profile happens in later disease stages [41, 45], just where we observed the induction of HLA-G expression.

A recent study by Nikolova et al. [57] demonstrated increased cell surface expression of ILT2/CD85j in circulating Sézary cells. Sézary syndrome is an aggressive form of cutaneous T-cell lymphoma characterized by erythroderma, lymphadenopathy, and the presence of CD4⁺CD45RO⁺ Sézary cells in peripheral blood [58]. ILT2 differs from other KIRs by the virtue of its distribution on phagocytic and antigen-presenting cells such as monocyte, macrophages, dendritic cells, and B lymphocytes [59, 60]. ILT2 is also expressed on some of the peripheral NK and T cells [61], in particular on CD8⁺
T cells with memory/effector phenotype [62]. Nikolova et al. [57] found that ILT2-expressing Sézary cells, as compared with autologous reactive CD4+ lymphocytes, are resistant to CD3 monoclonal antibody-induced cell death. It is suggestive that the expression of inhibitory receptors may enable survival of the malignant clone by protecting them from activation-induced cell death, as has already been reported in normal circulating memory/effector CD8+ T cells expressing ILT2 and other KIRs [62].

In one of our previous reports, we described that a particular subgroup of cutaneous lymphomas, cytotoxic CD8+ and CD56+ lymphomas express HLA-G ligand, ILT2 [63]. Cytotoxic lymphomas represent rare entities that have been recently recognized and characterized [64, 65]. These lymphomas often characterize aggressive clinical course with dubious prognosis. In a follow-up study, we could demonstrate HLA-G expression in 2 of 3 lymphomas of CD56+/CD4+ type and in all lymphomas of CD8+ type (manuscript in preparation). The expression of IL-10 matched the expression of HLA-G. IL-10 was implicated to affect the proliferation and/or cytotoxic phenotype in an autocrine manner in nasal NK-cell lymphomas [66]. Together with expression of different KIRs, IL-10 induced HLA-G upregulation could account for growth/survival advantage of the dominant clone and contribute to aggressive phenotype of these lymphoma entities.

Epithelial Skin Cancers

Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin represent the most common malignancies in the white human population worldwide [67, 68]. These two cancer types constitute around 95% of all NMSCs (75% BCC and 25% SCC) accounting for more than 1 million new patients each year [68]. Among epithelial tumors, BCC and SCC are unique with respect to their biologic behavior. They grow slowly and rarely metastasize. As discussed in the Introduction, sun exposure represents one of the major causative factors for the development of these tumors. The mutated p53 gene, a “UVR fingerprint,” is found in more than 90% of SCC and in most BCC [69]. These tumors are frequently surrounded by a varying degree of inflammatory infiltrate [70–72]. Even though inflammatory cells in BCC and SCC commonly express activation markers (e.g., IL-2 receptor, HLA-DR, perforin) the lysis of tumor cells fails to happen [67]. One of the explanations may be in the fact that tumor cells in BCC and SCC often produce immunosuppressive cytokines such as IL-10 [73–75], which then in context of cumulative UVR exposure (see Introduction) results in impairment of cell-mediated immunity. Given the fact that these tumors reside in the skin “untouched” by immune response for many years and that they so rarely give rise to metastases, implies that they have developed special strategies to be almost invisible to local immune watchers. Despite existing reports on alterations of HLA class I antigens in BCC and SCC, total loss of HLA class I molecules seems to be a rather rare event (in less than 10% of patients) [67]. HLA-G is once again a good candidate to provide tumor immune stealthiness. We conducted a preliminary study to evaluate HLA-G protein expression in primary BCCs by immunohistochemistry (manuscript in preparation). HLA-G protein was expressed to a greater extent in aggressive (morpheaform BCC) than in nonaggressive, superficial, and nodular BCC.

In their recent study Aractingi et al. [76] investigated the possibility of HLA-G upregulation in different malignant and premalignant skin lesions in renal transplant recipients. As mentioned previously, these patients have an increased risk to develop malignant tumors. Skin tumors (most frequently SCC) develop in sun-exposed areas, reaffirming the role of UVR in their pathogenesis [8, 11]. Following transplantation, IL-10 producing phenotype is associated with lower rejection rate providing a long-term protective effect [77]. From the cytokine aspect, once again there is a permissive environment for HLA-G upregulation. Given the beneficial role of HLA-G in mediating immune tolerance, HLA-G upregulation in the course of transplantation may be advantageous. Indeed, heart transplant recipients that had detectable HLA-G protein in the serum and myocardium presented with fewer acute rejection episodes and no chronic rejection [78, 79]. The question emerging addresses the possibility of HLA-G being a factor that additionally fosters tumor development after organ transplantation. Aractingi et al. [76] demonstrated HLA-G expression in different cutaneous neoplasms and preneoplastic conditions, but in none of the benign lesions in renal transplant recipients. An interesting observation was HLA-G immunoreactivity of proliferating keratinocytes in invasive and in situ SCC (Bowen’s disease) as well as in one case of actinic keratosis, which could not be detected in BCC, keratoacanthoma or in benign lesions. The authors observed weaker expression of HLA-G in SCC in situ then in invasive SCC. Besides UVR, human papillomaviruses (HPV) may also be cocarcinogenic in case of SCC, reaching PCR-positivity in up to 90% of transplant patients [8]. Viruses like human cytomegalovirus [80] or human immunodeficiency virus [81] can induce HLA-G expression on infected cells. Even though Aractingi and coworkers [76] did not assess their skin samples for HPV, it remains speculative whether HLA-G upregulation in the lesions belonging to SCC spectrum is a finding per se or a virally induced phenomenon.
CONCLUSION

Presented evidence implies that there may not be a direct association between HLA-G expression and the malignancy stage in melanoma as well as in nonmelanoma skin cancer. As with tumor immunosurveillance [1], immunoscape in general seems to be a very plastic process in a manner that is dependent on the tumor’s cell type of origin, tumorigenesis mechanism, anatomic localization, and mechanism of immunologic recognition. Thus, in some tumors HLA-G may be involved earlier in the course of malignant transformation with a decrease in later invasion stages, whereas in others it may be up-regulated later on during disease progression. It appears that HLA-G represents an independent characteristic of individual tumor rather than a rigid rule that can be applied to all. HLA-G expression should not be regarded as an exception either, given the heterogeneity of the tumors and the ways by which they have been “immunologically sculpted” [1]. By understanding these mechanisms, we will be able to delineate the nature and the factors responsible for HLA-G reactivation in cancer.

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