Heterogeneity of HLA-G Gene Transcription and Protein Expression in Malignant Melanoma Biopsies

Pascale Paul, Francisco Adrián Cabestró, Frédérique-Anne Le Gal, Iman Khalil-Daheb, Caroline Le Danff, Michel Schmid, Sylvaine Mercier, Marie-Françoise Avril, Jean Dausset, Jean-Gérard Guillet, and Edgardo D. Carosella

DSV-DRM, SRCHE/CEA, Commissariat à l’Energie Atomique, Centre Hayem, 75010 Paris, France. Phone: 33 (0)1 53 72 21 42; Fax: 33 (0)1 53 72 21 42; E-mail: paul@dsviff.cea.fr.

Abstract
Nonclassical MHC class I HLA-G antigen expression is tissue specific and is thought to play a role in tolerance of the semiallogeneic fetus by the maternal immune system. Ectopic expression of HLA-G by tumor cells provides them with an additional mechanism of escape from immunosurveillance by host cytotoxic effector mechanisms. The aim of this study was to assess the expression of nonclassical HLA-G antigens in ex vivo human melanoma biopsies.

HLA-G mRNA levels corresponding to both membrane-bound and soluble protein isoforms were analyzed in tumor specimens obtained from primary or metastatic melanomas of 23 patients. High levels of HLA-G transcription were detected in tumor specimens in 5 of 23 patients and found to be comparable in both lymph node and skin metastases. HLA-G mRNA transcript levels at tumor sites in 18 of these patients were compared with those in samples of their own healthy skin and were higher in the tumor tissue in 12 patients. Differential expression of mRNA transcripts corresponding to soluble and membrane-bound HLA-G was also observed in some tumor biopsies. HLA-G protein expression was detected in tumors that exhibited high levels of HLA-G transcription by immunofluorescence of frozen sections and Western blot analysis of both tumor and healthy skin biopsies, using anti-HLA-G-specific monoclonal antibodies. This work provides evidence that HLA-G gene transcription and protein expression can be up-regulated ex vivo in melanoma. Our finding that several of the tumors studied expressed high levels of HLA-G provides additional clues as to how a tumor can be selected in vivo to escape from cytotoxic antitumor responses, constituting a new parameter to be considered in the design of therapeutic approaches aimed at enhancing antitumor immune responses.

Introduction
Several studies have highlighted the importance of an effective T-cell immune response against specific human melanoma-associated antigens. Most tumors are still able to escape cell-mediated immune responses, progressing into metastatic disease. Several explanations have been proposed as to why the T-cell-mediated immune response is ineffective in eliminating melanoma cells, including defective helper T-cell function, inadequate costimulation of T cells, and deficient or reduced expression of MHC class I molecules caused by a defect in the class I heavy chain, the β2-microglobulin light chain, or the TAP peptide transporter (1–4). Melanoma therapies aimed at enhancing tumor immunogenicity have recently been the subject of much interest. Numerous studies suggest that disruption of KIR interactions with their MHC class I ligands can enhance the antitumor response (1, 6, 8, 9). CTL clones that express such inhibitory receptors can also be anergized in their MHC-restricted response to tumor cell clones that exhibit partial loss of HLA class I antigens, a phenomenon frequently observed in tumors (1, 17, 18). Heterogeneity of melanoma cell populations with regard to MHC class I expression is, thus, a key factor that must be considered when investigating the acquisition of metastatic potential by tumors as well as their capacity to escape from cytotoxic antitumor responses.

The variety of tumor cell phenotypes that influence the antitumor response may be even more complex. We have previously demonstrated that up-regulation of the nonclassical HLA-G gene may be observed in melanoma cell lines, in which it confers the ability to inhibit the NK cytotoxic function in vitro (19). HLA-G exists in multiple protein isoforms, including membrane-bound (HLA-G1, -G2, -G3, and -G4) and soluble (HLA-G5 and -G6) molecules, as a result of alternative splicing (20). Classical class I antigens are expressed at various levels in most tissues throughout the organism (21), whereas high levels of HLA-G gene transcription and protein expression are physiologically restricted to trophoblast cells during pregnancy. However, HLA-G protein expression has also been reported in thymic epithelial cells (22), first-trimester placental chorionic blood...
favor escape of HLA-G-expressing tumor cells from immunosurveillance to investigate how ectopic expression of HLA-G in tumors could indirectly favor such interactions. The capacity of HLA-G to function as an antigen that can inhibit cytotoxic NK effector function of the maternal immune system against its semiallogeneic fetus (30) has led us to investigate how ectopic expression of HLA-G in tumors could favor escape of HLA-G-expressing tumor cells from immunosurveillance (19). To further evaluate the biological relevance for patients of our previous observation of HLA-G expression in melanoma cell lines and to confirm that HLA-G is, indeed, detectable in vivo, we have analyzed transcription and protein expression were analyzed in primary and metastatic human melanoma biopsies obtained from 23 patients.

Table 1 Clinical and histopathological characteristics and levels of HLA-G transcripts encoding membrane-bound and soluble isoforms of surgically removed biopsies of 23 melanoma patients

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ARM</td>
<td>F</td>
<td>91</td>
<td>1.52</td>
<td>NED</td>
<td>HS</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>AUC</td>
<td>M</td>
<td>22</td>
<td>2.63</td>
<td>NED</td>
<td>SPT</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>BOU</td>
<td>F</td>
<td>44</td>
<td>UP</td>
<td>DD</td>
<td>SM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BOUV</td>
<td>F</td>
<td>52</td>
<td>2.26</td>
<td>AWD</td>
<td>SM</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CHO</td>
<td>F</td>
<td>58</td>
<td>2.59</td>
<td>NED</td>
<td>LNM</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>COL</td>
<td>F</td>
<td>50</td>
<td>2.04</td>
<td>NED</td>
<td>HS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>DAN</td>
<td>F</td>
<td>38</td>
<td>12</td>
<td>AWD</td>
<td>HS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DEL</td>
<td>M</td>
<td>39</td>
<td>5</td>
<td>NED</td>
<td>HS</td>
<td>+/−</td>
<td>−</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>DES</td>
<td>M</td>
<td>62</td>
<td>5.3</td>
<td>DOD</td>
<td>LNM</td>
<td>++</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>ESN</td>
<td>M</td>
<td>53</td>
<td>0.32</td>
<td>AWD</td>
<td>SM</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>ETI</td>
<td>F</td>
<td>50</td>
<td>1.8</td>
<td>AWD</td>
<td>LNM</td>
<td>++++</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+/−</td>
</tr>
<tr>
<td>FLI</td>
<td>M</td>
<td>60</td>
<td>0.7</td>
<td>NED</td>
<td>SPT</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>GOU</td>
<td>M</td>
<td>63</td>
<td>1.85</td>
<td>NED</td>
<td>HS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>GUI</td>
<td>M</td>
<td>59</td>
<td>0.55</td>
<td>NED</td>
<td>SPT</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>LOR</td>
<td>F</td>
<td>60</td>
<td>1.37</td>
<td>NED</td>
<td>HS</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>MAL</td>
<td>M</td>
<td>65</td>
<td>0.4</td>
<td>NED</td>
<td>LNM</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+/−</td>
<td>−</td>
</tr>
<tr>
<td>MAR</td>
<td>M</td>
<td>56</td>
<td>9</td>
<td>NED</td>
<td>SM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>MEL</td>
<td>M</td>
<td>64</td>
<td>5.4</td>
<td>NED</td>
<td>LNM</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>MEN</td>
<td>M</td>
<td>64</td>
<td>5.4</td>
<td>NED</td>
<td>HS</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>PAP</td>
<td>M</td>
<td>39</td>
<td>15</td>
<td>DOD</td>
<td>HS</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>PER</td>
<td>M</td>
<td>39</td>
<td>1.52</td>
<td>NED</td>
<td>LNM</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>SAV</td>
<td>M</td>
<td>77</td>
<td>2.2</td>
<td>NED</td>
<td>SPT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
<tr>
<td>TAP</td>
<td>M</td>
<td>73</td>
<td>6.42</td>
<td>NED</td>
<td>HS</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>VIG</td>
<td>M</td>
<td>59</td>
<td>10</td>
<td>NED</td>
<td>LNM</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
</tbody>
</table>

a B index, Breslow’s index; HS, healthy skin; SPT, skin primary tumor; HT, healthy s.c. tissue; SM, skin metastasis; HLN, healthy lymph node; LNM, lymph node metastasis; R, regression; NED, no evidence of disease; AWD, alive with disease; DOD, died of disease.

b At last evaluation.

c PCR (G.257–G3.U) indicates intensity of bands corresponding to the size of HLA-G1/G5, G2/G4, G3 transcripts as detected by RT-PCR, using primers that detect all HLA-G alternative transcripts. G.526–G.i4b corresponds to specific amplification of the HLA-G5-soluble isoform, −, lack of detection of HLA-G cDNA; +/−, faint or barely detectable signal; +, weak signal; + +, moderate signal; ++ +, strong signal; +++ +, very strong signal.

d vessel endothelial cells (23), and IFN-γ-treated mononuclear phagocytes (24). The HLA-G molecule exhibits very limited polymorphism and is thought to be able to interact with several described KIRs of the immunoglobulin superfamily including p49 (25), ILT2 and ILT4 (26, 27), expressed at the surface of NK cells but also on T-cell subsets, dendritic cells, B cells, and monocyte lineages. Direct interaction of HLA-G with the widely expressed CD94/NKG2A KIR receptors of the C-type lectin superfamily has been recently refuted (28, 29), with the demonstration that the nonclassical HLA-E molecule is the ligand for this receptor. The HLA-G peptide leader, like other class I peptides, allows HLA-E expression at the cell surface and can, thus, indirectly favor such interactions. The capacity of HLA-G to function as an antigen that can inhibit cytotoxic NK effector function of the maternal immune system against its semiallogeneic fetus (30) has led us to investigate how ectopic expression of HLA-G in tumors could favor escape of HLA-G-expressing tumor cells from immunosurveillance (19).
of their tumor was large enough to adequately assess Breslow’s thickness and to provide a sample for the study. Small-sized primary tumors were excluded. For skin and regional lymph node metastases, samples were provided by the pathologist if tissue samples were large enough to assess diagnosis and resection quality. After giving informed consent, 23 consecutive patients undergoing surgery at the Institut Gustave Roussy were included in the study.

No patient had received chemotherapy or systemic treatment during the 6 months prior to participation in the study. At the time they were included in the study, eight patients had an isolated primary melanoma, two patients had local recurrences within a 5-cm radius of the resected primary lesion, three patients had multiple skin metastases on the legs, eight patients had regional lymph node metastasis (including one who had an unresected primary tumor and another with in-transit metastasis), and two patients had disseminated disease for which they had undergone diagnostic (an untreated patient) or palliative regional lymph node resection. Tissue samples included healthy skin resected at the border of the surgical wound (n = 18), healthy lymph node (n = 1), healthy s.c. tissue (n = 1), skin primary tumor (n = 9), skin metastases (n = 9), lymph node metastases (n = 10), and a tumor regression area within a very large primary tumor (n = 1). Immediately after surgical excision, samples were removed from tumors under aseptic conditions, frozen in liquid nitrogen, and stored until RT-PCR and immunohistochemical staining could be carried out. Frozen blocks were cut into 5-μm sections, mounted on slides, and stained by the H&E method to evaluate the presence of tumor. Immunoperoxidase staining with S-100 and HMB45 antibodies (DAKO, Glostrup, Denmark) was used to confirm diagnosis as described (31). Patients characteristics at the time of sample inclusion are listed in Table 1.

**RT-PCR.** Total RNA was isolated from frozen samples by homogenization, using an ultraturrax device (IKA Labortechnik, Staufen, Germany) and RNA Now reagent (Biogentex, Seabrook, TX) according to manufacturers’ recommendations and checked by electrophoresis in a 1.5% agarose denaturing gel. One μg of RNA was reverse-transcribed to cDNA using oligo(dT) priming and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) at 42°C for 1 h. PCR was carried out in 100-μl volumes containing 200 μM each deoxynucleotide, 100 ng of each primer, 10 μl of 10× PCR buffer (Perkin-Elmer Corp.), and 2.5 units of Taq DNA polymerase (Perkin-Elmer Corp.). HLA-G-specific PCR amplifications were carried out using G.526 (5′-GGG TGA AGA GGA GAC TCT GCT GAC TAC AAG GGA) and G.i4F (5′-GGT CTG CAG GTT CAT TCT GTC) and G.i4b (5′-AAC GGA GGT GAA GTG GAG GG) and G.54b primers, detecting all alternatively spliced HLA-G5 transcripts. The PCR was conducted for 35 cycles, consisting of 1 min at 94°C, 90 s at 65°C (61°C for G5), and 2 min at 72°C, followed by a final extension of 7 min at 72°C. Coamplification of β-actin cDNA was carried out in each sample, using the β-actin amplifier set (Clontech) for 16 cycles, to compare RNA levels in samples. PCR specificity was confirmed by Southern blotting onto nylon membranes (Hybond N+; Amersham). Hybridization was carried out with the HLA-G-specific G.R (5′-GGT CTG CAG GTT CAT TCT GTC) and G.14F (5′-GAG GCA TCA TGT CTG TTA GG) probes, as described previously (19).

**Immunofluorescence Analysis of Tumor Biopsies.** Standard methods were used to carry out immunohistochemistry on frozen sections that had been fixed in acetone for 8 min and then rinsed in PBS and blocked in normal rabbit serum (DAKO) in PBS for 10 min. Samples were incubated with primary antibody for 1 h at room temperature, followed by incubation with a secondary FITC-conjugated antimouse immunoglobulin antibody for 15 min. Sections were counterstained with 4′,6-diamidino-2-phenylindole detection. A Nikon Diaphot 300 inverted microscope and a Nikon planochromat ×60 objective lens with a numerical aperture of 1.4 (Nikon Corp., Melville, NY) were used for fluorescent microscopy. The following antibodies were used: anti-MHC class I W6/32 (DAKO), antihuman melanoma HMB45 (DAKO), and anti-HLA-G 87G (which detects the HLA-G1 isoform; Ref. 32), a kind gift of Dan Geraghty, Fred Hutchinson Cancer Research Center (Seattle, WA).

**Western Blot Analysis of HLA-G Proteins in Tumor Samples.** When enough tumor specimen was available, proteins were extracted from melanoma tissues by homogenization in lysis buffer [25 mM Tris (pH 7.5), 150 mM NaCl, 10 mM EDTA, 1% NP40, and protease inhibitors (Complete; Boehringer Mannheim)], using an ultraturrax device. After removal of insoluble material by centrifugation at 10,000 × g for 20 min, the protein concentration was determined using a Bio-Rad (Richmond, CA) DC protein assay kit. Twenty-five-μg aliquots of total protein were separated by SDS-PAGE (10%) and transferred onto nitrocellulose membranes (Hybond-C extra; Amersham). After blocking, the membrane was probed with a 1:100 dilution of anti-HLA-G mAb 4H84 (22) directed against the α1 domain of the HLA-G molecule (kindly provided by M. McMaster). Antibody binding was detected using a peroxidase-conjugated secondary antibody (Sigma) and chemiluminescence (ECL+ detection kit; Amersham).
RESULTS

Detection of HLA-G mRNA Levels in Human Melanoma Biopsies. HLA-G mRNA levels in 28 melanoma specimens (described in Table 1) were obtained from the primary or metastatic tumors of 23 melanoma patients as well as healthy skin biopsies from 18 of the 23 patients, and tested by RT-PCR. In healthy skin samples, RT-PCR resulted in detection of basal low levels of the most abundant HLA-G1 transcripts, whereas the HLA-G2 transcripts were rarely detected, a pattern that is comparable to that described in peripheral blood lymphocytes (Fig. 1B; Refs. 33, 34). In 12 of 18 matched healthy and melanoma tissues, we detected a higher level of HLA-G transcription in tumor tissue than in the same patient’s healthy tissue (Figs. 1 and 2 and Table 1). Six of the 23 patients exhibited the same basal low level of HLA-G transcription in both tumor and healthy skin biopsies. A high level of HLA-G transcription, comparable to that observed in trophoblast-derived JEG-3 cells, was observed in five melanoma patients (VIG, CHO, ESN, GOU, and ETI; Fig. 1). The HLA-G transcription level observed at various tumor sites in the same patient were comparable, transcription activation being detected in both lymph node and s.c. skin metastases. Representative cases are shown in Fig. 1, and the results of analysis in other patients are summarized in Table 1.

Differential Expression of Soluble HLA-G Transcripts in Melanoma Biopsies. Specific RT-PCR analysis of soluble HLA-G5 RNA transcripts, using primers that amplify only the full-length secreted isoform, allowed us to detect differential transcription of soluble HLA-G5 transcripts in several patients (Fig. 2). Among the five melanomas that exhibited high levels of transcription, three (ESN, VIG, and ETI) had low or undetectable HLA-G5 transcript levels, whereas the two remaining tumor specimens (CHO and GOU) exhibited overall enhancement of all HLA-G transcripts. The three melanoma biopsies (MEN, PER, and LOR) that had lower HLA-G mRNA levels were also found to express high levels of the soluble HLA-G transcript. Representative cases are shown in Fig. 2, and the results of analysis in other patients are summarized in Table 1.

Detection of HLA-G Proteins in Human Melanoma Biopsies. HLA-G protein expression in melanoma tissues was evaluated by immunofluorescence, using mAb 87G, which is specific for the full-length HLA-G1 isoform. A correlation between high levels of HLA-G transcription and the expression of HLA-G protein was found in six melanoma biopsies obtained from four (ETI, CHO, ESN, and VIG) of the five patients in whom high levels of HLA-G mRNA were detected. For patient ESN, it was possible to analyze healthy skin as well as skin and lymph node metastases. HLA-G expression was selectively detected in both tumor sites but not observed in healthy skin (Fig. 3A). Biopsies of patients ETI (Fig. 3A) and CHO (data not shown), in whom a high level of HLA-G transcript was detected, were also positively stained by the anti-HLA-G antibody. Six biopsies of healthy skin obtained from melanoma patients as well as one sample of healthy skin obtained from normal breast plastic surgery, in which low or intermediate levels of HLA-G transcripts were detectable, were negative with the HLA-G antibody. Primary skin tumors of patients MEN and PER and skin metastasis of patient DES, all of which exhibited low levels of HLA-G transcription, were also negative for HLA-G protein expression. One of these tumor specimens (FLI) exhibited only moderate levels of HLA-G transcripts, similar to those found in healthy skin, and low HLA-G expression was reproducibly observed in this tumor by immunofluorescence (data not shown), indicating that low HLA-G transcription can still generate cell surface HLA-G protein expression. No immunofluorescence staining was detected with 87G in the healthy skin of this patient.

Western blot analysis, using the anti-HLA-G 4H84 mAb, revealed the presence of a Mr 39,000 band in protein extracts from lymph node metastatic tumor samples obtained from patients ESN, VIG, and ETI. This band is similar to one observed in the JEG-3 choriocarcinoma cell line, known to express the HLA-G1 protein. No signal corresponding to HLA-G was detected in healthy skin (Fig. 3B).

Levels of HLA-G Transcription and Protein Expression Measured in Healthy Skin, Skin Primary Tumor, Tumor Regression Site, and Melanoma Metastasis in the Same Patient Were Found to Correlate with the Proportion of Tumor Cells That Expressed Melanoma-associated Antigens. In one patient (VIG), it was possible to test healthy skin, primary cutaneous tumor, lymph node metastasis, and tumor regression within the skin primary tumor site specimens. Activation of HLA-G transcription was high in this patient’s primary and metastatic tumor specimens but low and comparable in both healthy skin and tumor regression sites (Fig. 4). No transcripts
corresponding to HLA-G5 were detected in these samples. HLA-G, melanoma antigen, and classical class I protein expression were analyzed in primary skin tumor, lymph node metastases, healthy skin, and a tumor regression site in this patient. Healthy skin from this patient was negative for 87G (Fig. 5A). Both the nodular area of primary melanoma and lymph node metastases exhibited strong homogeneous staining with anti-HLA-G, anti-classical HLA class I, and anti-melanoma-associated antigen antibodies (Fig. 5, B and C). No staining could be detected in a regressive area of the primary tumor in the same patient (Fig. 5D).

DISCUSSION
Constitutive and inducible HLA-G expression has been demonstrated in cultured tumor cell lines (19, 35, 36). HLA-G protein expression in surgically removed tumor biopsies has also been analyzed in a limited series of colorectal cancer, laryngeal carcinoma, kidney, thyroid, and breast cancer. Such expression remains controversial (36–38) and is probably underestimated because of the paucity of reagents able to detect HLA-G isoforms other than the full-length HLA-G1 membrane-bound isoform. The results obtained in this study are new findings demonstrating that primary and metastatic melanoma cells can express HLA-G in vivo. Various mechanisms can account for the escape of melanoma cells from immune destruction, including down-regulation of MHC class I molecules, FasL expression (39), and secretion of inhibitory factors such as interleukin 10 and transforming growth factor-β by tumor cells (40). Peptide-based vaccine and adoptive transfer clinical approaches do not always counterbalance the selection capacity of escape mechanisms provided by tumor cell phenotype heterogeneity. Apart from the commonly held opinion that tumors are poor stimulators of immune responses, recent evidence favors an active role of tumors in inducing T-cell tolerance (41).

Some phenotypical features, such as expression of the Fas ligand, are common to immune-privileged sites in which tolerance is established, such as trophoblast and the anterior chamber of the eye, where HLA-G expression is also physiologically restricted. We demonstrate here that ectopic HLA-G gene transcription and protein expression can be detected in melanoma cells ex vivo. Several genes, including MAGE, that exhibit testis- and placenta-restricted distribution are
activated in melanoma. These genes are thought to be activated through demethylation processes or by release from stringent genetic control, which could also trigger the expression of other genes, including HLA-G (42). We have shown that activation of HLA-G gene transcription and protein expression can occur in melanoma cells and could be considered a way the immune system is rendered ineffective against HLA-G-positive tumor subsets. This could occur by inhibiting the cytotoxic function of immune effector cells (19) through interaction with KIRs of both NK and T cells. Recent works show that the expression of immunoglobulin superfamily KIR receptors that interact with HLA-G is not restricted to NK cells. In addition to NK cells, the ILT2 receptor is expressed in T-cell subsets, B lymphocytes, and myelomonocytic cells (27, 43) and ILT4 is selectively expressed in monocytes, macrophages, and dendritic cells (26).

Other as yet unidentified immunological functions of HLA-G expression in melanoma, such as enhancement of HLA-E expression (28, 44) and interaction with other activating or inhibitory receptors expressed on various immunocompetent cells, may, thus, become evident in the near future.

The capacity of tumors to secrete the soluble HLA-G isoform could also favor their spread. Soluble HLA-G5, similar in structure to soluble HLA-G with the severity of disease or with metastatic potential, this work clearly establishes the fact that such HLA-G expression in melanoma is, indeed, observed in vivo. Additional follow-up studies of HLA-G expression status in primary tumors regarding their capacity to metastasize will permit evaluation of this parameter as a facilitator of tumor progression.

Analysis of the HLA-G status of tumors before and during treatment and the design and development of pharmacological tools that selectively down-regulate HLA-G expression at the surface of HLA-G-positive tumor cells should help to provide complementary therapeutic approaches in the treatment and follow-up of human melanoma.

ACKNOWLEDGMENTS

We thank Daniel Geraghty, Susan Fisher, and Michael McMaster for providing us with HLA-G antibodies. We also thank Christelle Dolfner for her technical assistance. We are grateful to Pierre Duviillard and Alvaro Margulis (Department of Pathology and Surgery, Institut Gustave Roussy, Villejuif, France) for providing us with histopathology reports and tumor sample biopsies. We thank Noah Hardy, Nathalie Rouas-Freiss, and Julie-Ann Gavigan for reading and correcting the manuscript. We express our thanks to Prof. R. Gómez-Lus for his interest in this work.

REFERENCES


Heterogeneity of HLA-G Gene Transcription and Protein Expression in Malignant Melanoma Biopsies


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/8/1954

Cited articles
This article cites 54 articles, 16 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/8/1954.full#ref-list-1

Citing articles
This article has been cited by 17 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/8/1954.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/59/8/1954.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.