HLA-G Immunoreactivity Is Specific for Intermediate Trophoblast in Gestational Trophoblastic Disease and Can Serve as a Useful Marker in Differential Diagnosis

Gad Singer, M.D., Robert J. Kurman, M.D., Michael T. McMaster, Ph.D., and Ie-Ming Shih, M.D., Ph.D.

HLA-G is a nonclassical MHC class I antigen that has been shown to be a specific marker for normal intermediate trophoblast (IT). In this study HLA-G immunoreactivity assessed with an HLA-G specific antibody (4H84) was detected in all 14 cases of choriocarcinoma, 14 placental site trophoblastic tumors, 13 epithelioid trophoblastic tumors, 16 placental site nodules, and nine exaggerated placental sites. In contrast, HLA-G immunoreactivity was not detected in 34 nontrophoblastic uterine neoplasms. HLA-G immunoreactivity was present in all the IT cells of exaggerated placental sites and placental site trophoblastic tumors and in 70–100% of IT cells in placental site nodules and epithelioid trophoblastic tumors. The pattern of distribution of HLA-G in different subpopulations of IT confirms the relationship of various trophoblastic lesions to different types of IT (exaggerated placental site and placental site trophoblastic tumor to implantation site IT and placental site nodule and epithelioid trophoblastic tumor to chorionic-type IT) and suggests that choriocarcinoma is related to villous-type IT because the majority of mononucleate cells in this neoplasm were HLA-G immunoreactive. In conclusion, HLA-G immunoreactivity appears to be specific for IT in gestational trophoblastic disease and can serve as a useful marker in the differential diagnosis of these lesions.

Key Words: Trophoblast—HLA-G—Diagnosis.

HLA-G isoforms in a variety of gestational trophoblastic lesions and a wide range of common uterine neoplasms to assess its utility for the differential diagnosis of gestational trophoblastic lesions. Complete and partial moles were not studied because expression of HLA-G in molar placentas has been previously reported,3 and the distinction of nontrophoblastic tumors from molar placentas is not a problem.

MATERIALS AND METHODS

Tissue Samples

Formalin-fixed, paraffin-embedded tissue blocks from 10 normal first trimester placentas, 66 gestational trophoblastic lesions, and 34 miscellaneous nontrophoblastic uterine neoplasms were retrieved from the archival files of the Gestational Trophoblastic Disease Tissue Bank and the Surgical Pathology files of the Johns Hopkins Hospital. Most of the trophoblastic lesions were sent in consultation to one of the authors (R.J.K.). These trophoblastic lesions included 14 choriocarcinomas, 14 placental site trophoblastic tumors, 13 epithelioid trophoblastic tumors, 16 placental site nodules, and nine exaggerated placental sites. The nontrophoblastic uterine tumors included 10 invasive cervical squamous cell carcinomas, nine poorly differentiated endometrial carcinomas, nine low-grade endometrial stromal sarcomas, one malignant mixed mesodermal tumor, one leiomyosarcoma, one epithelioid leiomyosarcoma, one typical leiomyoma, and two epithelioid leiomyomas.

Immunohistochemistry

After deparaffinization, tissue sections (5 μm) were steamed in a citrate buffer for 5 minutes to facilitate antigen retrieval. The slides were then incubated with the supernatant of the 4H84 monoclonal antibody (IgG1, provided by Michael M. McMaster, 1:600)⁵ using the avidin-biotin peroxidase method. The reaction product was visualized with 3,3’-diaminobenzidine chromagen and the sections counterstained with 0.1% hematoxylin (Sigma, St. Louis, MO, USA), dehydrated, and mounted. Immunoperoxidase staining was performed with the automated Bio Tek-1000 immunostainer system (Bio Tek Solutions Inc., Santa Barbara, CA, USA). All procedures were performed according to the manufacturer’s protocols. A Mel-CAM (CD146) polyclonal antibody¹⁸ and an hPL monoclonal antibody were used for comparison with the staining pattern of HLA-G. The staining procedures for negative controls were the same except for the replacement of the primary antibody with a preimmunized mouse serum.

RESULTS

The immunostaining results of HLA-G in gestational trophoblastic lesions and a variety of uterine neoplasms are summarized in Table 1. All trophoblastic lesions, including choriocarcinomas, placental site trophoblastic tumors, epithelioid trophoblastic tumors, placental site nodules, and exaggerated placental sites examined, demonstrated HLA-G immunoreactivity in at least 70% of cells. A total of 100% of implantation site IT cells in placental site trophoblastic tumors, like their normal counterparts in the implantation site and exaggerated placental site, showed strong membranous and cytoplasmic staining for HLA-G (Figs. 1, 2). In exaggerated placental site the IT cells infiltrate the endomyometrium as single cells or cords, often in a linear pattern, whereas in placental site trophoblastic tumors the cells form confluent masses (Figs. 1, 2). This feature can assist in the differential diagnosis of these lesions. In contrast to implantation site IT, the percentage of HLA-G-positive chorionic-type IT cells in the chorion laeve of the normal placenta ranged from 60% to 70%. Specifically, IT cells that contained eosinophilic cytoplasm were immunoreactive for HLA-G, but IT cells

<table>
<thead>
<tr>
<th>Lesions</th>
<th>No. of cases</th>
<th>No. of HLA-G immunoreactive IT cells within a lesion</th>
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</thead>
<tbody>
<tr>
<td>Choriocarcinoma</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Placental site trophoblastic tumor</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Epithelioid trophoblastic tumor</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Placental site nodule</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Exaggerated placental site</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Non-trophoblastic uterine tumors*</td>
<td>34</td>
<td>0</td>
</tr>
</tbody>
</table>

* These included 10 invasive cervical squamous cell carcinomas, 9 poorly differentiated endometrial carcinomas, 9 low-grade stromal sarcomas, 1 malignant mixed mesodermal tumor, 2 leiomyosarcomas, and 3 leiomyomas.
with clear cytoplasm were always negative (Fig. 3). In epithelioid trophoblastic tumors, 70–100% of IT cells were positive for HLA-G (Fig. 4) and in placental site nodules 70–90% of IT cells were positive for HLA-G (Fig. 5). As in the chorion laeve, the HLA-G-positive IT cells in epithelioid trophoblastic tumors and placental site nodules were nearly always those with eosinophilic cytoplasm; the cells with clear cytoplasm were rarely positive. The immunostaining pattern of HLA-G was compared with hPL and Mel-CAM (CD146) in the placental site nodules. HLA-G was diffusely expressed in IT cells, whereas hPL and Mel-CAM expression was only focal (Fig. 5). The staining intensity of HLA-G in IT cells in placental site trophoblastic tumor and epithelioid trophoblastic tumor appeared to be comparable with normal IT cells. In choriocarcinoma HLA-G was localized in mononucleate cells but not in syncytiotrophoblast cells (Fig. 6A, B). The HLA-G-positive cells were also immunoreactive to Mel-CAM, which is specifically expressed in IT cells in choriocarcinomas. In contrast to IT cells, myometrial, endometrial, endothelial, and endometrial stromal cells as well as lymphocytes were negative for the HLA-G antibody. HLA-G immunoreactivity was not detected in any of the nontrophoblastic uterine neoplasms (Table 1).

**DISCUSSION**

In this study HLA-G immunoreactivity was expressed in all gestational trophoblastic lesions, including exaggerated placental site, placental site nodule, placental site trophoblastic tumor, epithelioid trophoblastic tumor, and choriocarcinoma, but not in nontrophoblastic uterine tumors. The detailed distribution of HLA-G in a variety of tumors outside the female genital tract has been previously reported.2,7,9 Briefly, the vast majority of these nontrophoblastic tumors fail to express HLA-G.2,9 Exceptions include melanoma, renal cell carcinoma, and large cell carcinoma of the lung, which in some cases...
demonstrate scattered focal immunostaining for HLA-G. This restricted expression of HLA-G in extrauterine tumors together with our findings showing an absence of HLA-G immunoreactivity in nontrophoblastic uterine tumors and normal uterine tissues suggests that HLA-G can be a very useful marker in the diagnosis of trophoblastic tumors and tumor-like lesions.

In this study HLA-G immunoreactivity was identified in IT but not in cytotrophoblast and syncytiotrophoblast. Compared with other trophoblastic markers, HLA-G appears to be more sensitive and specific for IT. Tables 2 and 3 summarize the immunohistochemical findings of several antibodies in the different trophoblast subpopulations and related lesions. For example, β-hCG is exclusively expressed in syncytiotrophoblast and is useful in the diagnosis of choriocarcinoma, but in exaggerated placental sites, placental site nodules, placental site trophoblastic tumors, and epithelioid trophoblastic tumors, β-hCG expression is typically minimal or focal. Similarly, hPL is diffusely expressed in placental site trophoblastic tumors and exaggerated placental sites, but it is only focally (<10%) expressed in choriocarcinoid-type IT cells in placental site nodules and epithelioid trophoblastic tumors. Mel-CAM (CD146) is more sensitive than hPL as it is expressed in 100% of implantation site IT in exaggerated placental sites and placental site trophoblastic tumors but is only focally expressed in placental site nodules and epithelioid trophoblastic tumors. PIAP lacks consistent staining in placental site nodules and is only weakly or not expressed in epithelioid trophoblastic tumors and implantation site IT. In contrast to these markers, HLA-G stains 70–100% of the IT cells in all of these lesions. HLA-G immunostaining, by highlighting the growth patterns of IT, can facilitate the distinction between a placental site trophoblastic tumor and an exaggerated placental site. In a placental site trophoblastic tumor the cells form confluent masses, whereas in an exaggerated placental site the IT cells infiltrate the endomyometrium as single cells and cords of cells. Other markers used in the differential diagnosis of trophoblastic lesions such as cytokeratin 18 and inhibin-α are expressed in a variety of other tumors besides trophoblastic lesions.

Analysis of the HLA-G expression in the placenta and in trophoblastic lesions also sheds light on the pathogen-
esis of trophoblastic neoplasms. The diffuse expression of HLA-G in the trophoblast of trophoblastic columns (Fig. 6C) and the implantation site in all stages of placentation is consistent with the previous report1 and supports the view that trophoblastic cells in the trophoblastic columns are a subpopulation of IT cells, not cytotrophoblast, as the latter are negative for HLA-G. Thus, although choriocarcinoma has been traditionally described as being composed of cytotrophoblast and syncytiotrophoblast, the presence of HLA-G, a specific marker of IT, in the vast majority of mononucleate cells in choriocarcinoma indicates that these cells are IT, not cytotrophoblast. This interpretation is supported by our previous study showing that the majority of cells in choriocarcinoma are positive for Mel-CAM, which is also expressed by IT but not cytotrophoblast.11 The arrangement of trophoblastic cells in choriocarcinoma (Fig. 6A, B) bears a close resemblance to the arrangement of trophoblast in the trophoblastic columns (especially from complete hydatiform moles) with cytotrophoblast typically differentiating into IT (villous-type) and then syncytiotrophoblast. Besides, similar to choriocarcinoma, the IT cells in the trophoblastic columns of complete hydatidiform moles strongly and diffusely express HLA-G.3,8 Thus, we speculate that choriocarcinoma develops as a result of transformation of villous-type IT (in normal placenta or complete hydatidiform moles) that differentiates into syncytiotrophoblast. It is also conceivable that cytotrophoblast differentiates directly into syncytiotrophoblast, but the former pathway is more likely as choriocarcinomas contain only a small number of cytotrophoblastic cells. This pathway of differentiation for choriocarcinoma contrasts to that of placental site trophoblastic tumor in which villous-type IT differentiates into implantation site IT or in epithelioid trophoblastic tumor where villous-type IT differentiates into chorionic-type IT.14

The distribution of HLA-G immunoreactivity in chorionic-type IT, as described in this study in conjunction with previous studies,22 suggests that this type of trophoblast is comprised of two distinct cell populations, one with clear cytoplasm and one with eosinophilic cytoplasm. The finding that HLA-G immunoreactivity is confined to the chorionic-type IT cells with eosinophilic cytoplasm but is rarely detected in cells with clear cytoplasm is similar to our previous reports showing that...
Mel-CAM (CD 146) and hPL are most often localized in this IT subpopulation, although in a much lower percentage of cells. The same differential HLA-G expression is present in placental site nodules and epithelioid trophoblastic tumors, thus accounting for the variable proportion of HLA-G-positive IT cells in these lesions, and provides further support for the view that placental site nodule and epithelioid trophoblastic tumor are related to the IT cells in the chorion laeve\textsuperscript{13,17} (Table 3).

Because trophoblastic lesions are gestational in origin, they contain paternal antigens and represent semi-allografts to the patient. Recent advances in reproductive immunology have shed light on the molecular mechanisms underlying the immunologic tolerance of trophoblastic cells. Thus, it appears that the expression of HLA-G plays a role in the development of immunotolerance of IT cells. This could possibly account for the long latency periods that have been observed for choriocarcinoma and for other trophoblastic lesions derived from IT, such as placental site nodules and epithelioid trophoblastic tumors, which have been discovered many years after patients have had tubal ligations.\textsuperscript{13,15,17,24}

In conclusion, we have shown that an HLA-G-specific antibody (4H84) specifically stains IT cells in normal

\begin{table}[h]
\centering
\caption{Immunohistochemical features of the different trophoblastic cell populations in the normal early placenta}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
 & Cytotrophoblast & Villous IT & Implantation site IT & Chorionic-type IT & Syncytiotrophoblast \\
\hline
HLA-G & – & +++ & +++ & +++\textsuperscript{a} & – \\
β-hCG & – & – & – & – & – \\
hPL & – & –/++\textsuperscript{c} & +++ & –/+ & +++ \\
Mel-CAM & – & –/++++\textsuperscript{c} & +++ & –/+ & – \\
PLAP & – & – & – & +++ & – \\
Ki-67 index\textsuperscript{d} & 25–50% & >90% & 0 & 3–10% & 0 \\
\hline
\end{tabular}
\textsuperscript{a} Mainly in cells with eosinophilic cytoplasm. \\
\textsuperscript{b} In multinucleated IT. \\
\textsuperscript{c} Toward the distal end. \\
\textsuperscript{d} Ki-67 immunointensity decreases from the base to the tip of the trophoblastic column in normal placenta. \\
β-hCG, human chorionic gonadotropin; hPL, human placental lactogen; Mel-CAM, melanoma cell adhesion molecule (CD146); PLAP, placental alkaline phosphatase. +++ denotes semiquantitative scoring of proportion of cells showing a positive reaction.
\end{table}
placentas and trophoblastic lesions in paraffin sections. Although HLA-G appears to be an excellent marker for IT in trophoblastic lesions, it should be noted that rare metastatic tumors to the uterus may also express HLA-G, but the distribution is focal in contrast to the diffuse expression of HLA-G in trophoblastic lesions. Thus, this antibody can be used in combination with other antibodies, such as those that react with inhibin-α and CK18 in the differential diagnosis of gestational trophoblastic lesions from nontrophoblastic tumors. A combination of Mel-CAM, hPL, and Ki-67 can then be used to further distinguish among different trophoblastic lesions.16

Table 3.

<table>
<thead>
<tr>
<th>Immunohistochemical features of the different trophoblastic cell populations in gestational trophoblastic tumors and tumor-like lesions</th>
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</thead>
<tbody>
<tr>
<td><strong>Implantation site IT lesions</strong></td>
</tr>
<tr>
<td>Choriocarcinoma</td>
</tr>
<tr>
<td>HLA-G</td>
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<tr>
<td>β-hCG</td>
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<tr>
<td>hPL</td>
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<td>Mel-CAM</td>
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<tr>
<td>PLAP</td>
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<tr>
<td>Ki-67 index</td>
</tr>
</tbody>
</table>

*Mainly in multinucleate IT.

**Mainly in cells with eosinophilic cytoplasm.

IT, intermediate trophoblast; ST, syncytiotrophoblast; β-hCG, human chorionic gonadotropin; hPL, human placental lactogen; Mel-CAM, melanoma cell adhesion molecule (CD146); PLAP, placental alkaline phosphatase; +++ denotes semiquantitative scoring of proportion of cells showing a positive reaction. + = <25%; ++ = 25–50%; +++ = >50–75%; +++⁺⁻ = >75%.

References