Metastatic Trophoblastic Disease after an Initial Diagnosis of Partial Hydatidiform Mole

Genotyping and Chromosome In Situ Hybridization Analysis

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BACKGROUND. Hydatidiform mole (HM) is classified into partial (PHM) and complete (CHM) subtypes according to histopathologic and genetic criteria. Traditionally, it is believed that PHM carries a better prognosis and rarely develops metastasis. However, making a distinction between PHM and CHM using histologic criteria alone may be difficult.

METHODS. The authors used fluorescent microsatellite genotyping following laser-capture microdissection and chromosome in situ hybridization (CISH) to perform a genetic analysis of six patients with histologically diagnosed PHM who subsequently developed metastastic gestational trophoblastic neoplasia.

RESULTS. Patients ranged in age from 25 years to 44 years (mean, 33.2 years). The gestational age of the molar pregnancies varied from 6 weeks to 20 weeks. All six patients had pulmonary metastases, with additional liver metastasis in two patients. Among the six patients with histologically diagnosed PHM, it was found that four patients had a diploid karyotype and no maternal alleles; thus, their neoplasms actually were CHM. Maternal genome was detected in the remaining two patients consistent with a biparental origin, and these patients had a triploid karyotype. CISH findings in all patients correlated with the genotyping findings. Triploid HM had maternally derived alleles, whereas diploid HMs were purely androgenetic.

CONCLUSIONS. In the current study, which may be the largest series of genetically analyzed metastatic PHMs to date, the difficulty of histologic distinction between PHM and CHM was confirmed. Molecular analysis may help to refine the classification of HM. Although the current findings support the belief that most aggressive trophoblastic diseases are derived from CHM, a small number of PHMs do progress to metastatic disease. Thus, the current study reaffirmed that all patients with HM should be followed closely irrespective of histologic subclassification.

Gestational trophoblastic disease (GTD) is a disease of the trophoblastic tissue that includes a heterogeneous variety of lesions with variable degrees of neoplastic changes. GTD includes benign hydatidiform mole (HM), invasive mole, choriocarcinoma, placental site trophoblastic tumor, and epithelioid trophoblastic tumor.1-2 HM is the most common type and is characterized by significant hydropic enlargement and variable trophoblastic hyperplasia. Although the majority of HMs spontaneously regress after suction evacuation, some may develop gestational trophoblastic neoplasia (GTN) and thus require chemotherapy. Metastases may develop occasionally. HM can be subclassified into complete

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(CHM) and partial (PHM) subtypes based on morphologic, pathologic, and genetic differences. In general, it is believed that CHM carries a much greater risk of developing GTN. Nevertheless, PHMs with aggressive sequelae occasionally are reported.

Although CHM and PHM traditionally are considered to be histologically distinguishable, the diagnosis of HM based on morphologic differences alone has the problem of interobserver and intraobserver variability. Techniques that make use of the genetic difference between PHM and CHM have been developed to assist in making the differential diagnosis. Genetically, CHMs usually are diploid and androgenetic in origin, with all 46 chromosomes originating entirely from the sperm. In contrast, most PHMs are triploid and are formed by dispermic fertilization of a normal ovum. Thus, both PHMs and CHMs possess two sets of paternal haplotypes (diandric) in the genome. Nevertheless, unlike CHMs, which have no maternal contribution, PHMs have one set of chromosomes contributed by the maternal ovum.

In the current study, retrospective genetic analysis was performed for six patients with rare cases of histologically diagnosed PHM from which metastatic disease subsequently developed. To our knowledge, it is likely that the current study is the largest series to date involving genetic analysis of metastatic PHMs. We used fluorescent microsatellite genotyping to identify the parental origin of the molar tissue; this technique, together with chromosome in situ hybridization (CISH), can be used to identify genuine metastatic PHMs.

### MATERIALS AND METHODS

#### Patients

Patients with histologically diagnosed PHM who subsequently developed metastatic GTN were identified from the clinical records of Department of Obstetrics and Gynecology and the Department of Pathology at Queen Mary Hospital, The University of Hong Kong (Hong Kong, China), between 1989 and 1996. After suction evacuation of the molar pregnancy, patients were monitored in a standard fashion, including serial serum and urinary human chorionic gonadotrophin (β-hCG) assays. In our center, GTN was suspected when β-hCG levels remained the same for 4 weeks or if there was a rising β-hCG level for 3 consecutive weeks when pregnancy was excluded. Patients with suspected GTN were evaluated for evidence of metastatic disease.

Most patients with metastatic PHM in the current study were referred to our institution. The diagnosis of metastatic trophoblastic disease was based on clinical, serologic, and radiologic evidence. No tissue diagnosis of the metastatic lesion had been obtained. The original pathologic diagnosis had been made based on histologic criteria on hematoxylin and eosin (H&E)–stained sections from each patient. Partial mole was diagnosed when there was a certain degree of fetal development, including nucleated red blood cells, or when the hydropic change was observed in only a portion of the chorionic villi. Trophoblastic proliferation usually was focal.

Formalin-fixed, paraffin-embedded tissue samples containing both maternal endometrium and molar villi were obtained successfully by uterine curettage in six patients. The clinical and pathologic features of all patients are listed in Table 1. Patients ranged in age from 25 years to 44 years (mean, 33.2 years). The gestational age of the molar pregnancies varied from 6 weeks to 20 weeks. All patients had serum hCG levels that remained stationary for at least 4 weeks. Investigations included chest X-ray, pelvic and hepatic arteriograms, and magnetic resonance imaging.

### TABLE 1

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Patient age (yrs)</th>
<th>Wks of gestation</th>
<th>Initial pathologic diagnosis</th>
<th>Presence of fetal tissue</th>
<th>Mos between diagnosis and CT</th>
<th>Treatment risk factor</th>
<th>Site(s) of metastases</th>
<th>Mos between diagnosis and metastasis detection</th>
<th>Presence of maternal genome (genotyping)</th>
<th>Ploidy (CISH)</th>
<th>Reviewed pathologic diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>36</td>
<td>11</td>
<td>PHM</td>
<td>No</td>
<td>9</td>
<td>M</td>
<td>Lung</td>
<td>0</td>
<td>No</td>
<td>Diploid</td>
<td>CHM</td>
</tr>
<tr>
<td>49</td>
<td>44</td>
<td>10</td>
<td>PHM</td>
<td>No</td>
<td>2</td>
<td>H</td>
<td>Lung, liver</td>
<td>0</td>
<td>No</td>
<td>Diploid</td>
<td>CHM</td>
</tr>
<tr>
<td>50</td>
<td>31</td>
<td>20</td>
<td>PHM</td>
<td>No</td>
<td>2</td>
<td>H</td>
<td>Lung, liver</td>
<td>0</td>
<td>Yes</td>
<td>Triploid</td>
<td>PHM</td>
</tr>
<tr>
<td>52</td>
<td>35</td>
<td>10</td>
<td>PHM</td>
<td>No</td>
<td>3</td>
<td>M</td>
<td>Lung</td>
<td>0</td>
<td>Yes</td>
<td>Triploid</td>
<td>PHM</td>
</tr>
<tr>
<td>54</td>
<td>25</td>
<td>6</td>
<td>PHM</td>
<td>No</td>
<td>2</td>
<td>M</td>
<td>Lung</td>
<td>4</td>
<td>No</td>
<td>Diploid</td>
<td>CHM</td>
</tr>
<tr>
<td>55</td>
<td>28</td>
<td>9</td>
<td>PHM</td>
<td>No</td>
<td>3</td>
<td>M</td>
<td>Lung</td>
<td>0</td>
<td>No</td>
<td>Diploid</td>
<td>CHM</td>
</tr>
</tbody>
</table>

CT: chemotherapy; CISH: chromosome in situ hybridization; PHM: partial hydatidiform mole; CHM: complete hydatidiform mole; M: medium risk; H: high risk.
images of the brain for patients with lung metastasis. All patients had lung metastasis that varied in size from 5.0 mm to 1.5 cm. Two patients had liver metastasis, as evidenced on hepatic arteriograms, that ranged in size from 6 mm to 10 mm. Two patients were treated with methotrexate, two patients were treated with actinomycin-D and methotrexate, and two patients were treated with modified hydroxurea, vincristine, methotrexate, actinomycin-D, and cyclophosphamide chemotherapy regimens. All patients responded well to chemotherapy and remained free of disease for 8–14 years.

DNA Preparation
For each specimen, the maternal decidua and molar villi were microdissected separately from 6 μm, H & E–stained sections using the Arcturus PixCell® II LM 200 Laser Capture Microdissection (LCM) System (Arcturus Engineering, Mountain View, CA).22 DNA then was prepared from the laser microdissected tissue according to the manufacturer’s protocol.24 In brief, DNA from the dissected cells on the Capsure™ HS LCM cap (Arcturus Engineering) was extracted by bringing the cap surface into contact with 50 μL digestion buffer containing 0.04% proteinase K; 10 mM Tris-HCl, pH 8.0; 1 mM ethylenediamine tetraacetic acid (EDTA); and 1% Tween 20 (Sigma Chemical Co., St Louis, MO). After overnight incubation at 37 °C, the extraction tube was centrifuged, and the fluid was heated to 95 °C for 10 minutes to inactivate the proteinase K.

Polymerase Chain Reaction
In each case, 3 μL of DNA from both maternal and molar tissue samples were amplified with 6 pairs of primers that flanked polymorphic microsatellite repeat sequences on 5 different chromosomes (Table 2).22 The loci included D3S1358, D5S818, D13S267, D17S1322, D17S855, and vWA.25 Polymerase chain reaction (PCR) analysis was performed in a 10 μL reaction volume containing 1X PCR buffer (10 mM Tris-HCl and 50 mM KCl, pH 8.3), 2.0 mM or 2.5 mM magnesium chloride, 250 μM of each deoxyribonucleoside triphosphate, 0.4 μM each of the forward and reverse primers, and 0.6 units of AmpliTaq Gold® (Applied Biosystems, Foster City, CA). One primer from each primer pair was labeled with a fluorescent dye—fluorescein, hexachlorofluorescein, or carboxytetramethylrhodamine. The PCR reaction consisted of an initial denaturation step at 95 °C for 12 minutes; followed by 39 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 1 minute, with extension at 72 °C for 40 seconds; and a final extension at 72 °C for 10 minutes using the GeneAmp® PCR System 9600 (Applied Biosystems). After amplification, 2.5 μL of each PCR reaction product underwent electrophoresis in a 2% agarose gel to assess the yield.

Microsatellite Genotyping
The PCR products from each sample were then diluted according to the yield. After diluting with an appropriate volume of a mixture of formamide and blue dextran/EDTA containing a carboxy-x-rhodamine-labeled internal size standard, the PCR products were denatured at 95 °C, placed on ice to chill, and then separated by electrophoresis in a 5% denaturing polyacrylamide gel using the ABI PRISM 377 DNA Sequencer (Applied Biosystems).22 Data analysis and fragment sizing of the microsatellite polymorphism were performed using ABI PRISM GeneScan® analysis software (Version 3.1; Applied Biosystems) and Genotyper® fragment analysis software (Version 2.5; Ap-
plied Biosystems). The allele sizes of microsatellite polymorphisms found in the molar villi then were compared with the corresponding allele sizes observed in the maternal tissue.

**CISH**

The ploidy of each HM was studied with CISH. DNA probes specific for the pericentromeric regions of chromosome 11 (D11Z1), chromosome 16 (D16Z1), chromosome Y, DYZ5 (American Type Culture Collection, Rockville, MD), and chromosome X (pBamX5; a generous gift from Dr. A. H. N. Hopman; Department of Molecular Cellular Biology and Genetics, University of Limburg, Maastricht, The Netherlands) were used. All DNA probes were labeled with biotin 11-deoxyuridine triphosphate using the standard nick-translation method (Boehringer Mannheim, Indianapolis, IN).

Paraffin sections (thickness, 5 μm) were mounted on 2% aminopropyltriethoxysilane-coated slides. After deparaffinization, the slides were incubated with proteinase K (250–500 μg/mL) and 0.1% Triton X-100 at 50 °C. The labeled probes were added to the hybridization mix (60% formamide, 10% dextran sulfate, 2× standard saline citrate) and applied to the tissue sections at probe concentrations of 1 ng/μL of hybridization mixture. Denaturation was performed at 90 °C, followed by overnight hybridization at 37 °C with intermittent agitation. Immunohistochemical analysis was performed using avidin, biotinylated mouse antiantiavidin, rabbit antimouse peroxidase, and 3,3′-diaminobenzidine (Dakopatts Ltd., High Wycombe, United Kingdom). Hydrogen peroxidase was used to visualize peroxidase activity.

In each of the sections that was hybridized with individual, chromosome-specific DNA probes, at least 200 nuclei were assessed by independent observers (W.-C.X. and A.N.Y.C.). A gain in chromosome 11 or chromosome 16 was inferred when ≥ 15% of the tumor cells displayed 3 signals or > 3 signals.

**RESULTS**

**Fluorescent Microsatellite Genotyping**

In the interpretation of genotyping results, the absence of maternal genome in the HM was indicated by the exclusion of maternal allele(s) in the molar tissues. Alleles in the molar tissue that were not found in the maternal tissue were interpreted as being paternal. In contrast, HMs with one or more maternal alleles definitively demonstrated by microsatellite markers were interpreted as being biparental. Four of six metastatic moles (Patients 47, 49, 54 and 55) exhibited genotyping profiles that indicated an absence of maternal contribution (Fig. 1), while a maternal contribution was observed in two moles (Patients 50 and 52).

**CISH**

From CISH studies, triploidy was inferred in two moles (Patients 50 and 52), whereas the other four moles (Patients 47, 49, 54 and 55) exhibited a diploid composition (Fig. 2). No signal for the Y chromosome was documented in any of the six moles. In all moles, genotyping findings were correlated with CISH findings. Triploid HMs had maternally derived alleles, whereas diploid HMs were purely androgenetic (Table 1).

**Histology**

Histologic review, which included the two genetically biparental moles (Patients 50 and 52), did not reveal fetal tissue or nucleated red blood cells. The presence of two groups of chorionic villi (one group was more hydropic) was more conspicuous in the two true PHMs.

**Correlation between Histologic Diagnosis and Genotyping**

Genotyping and CISH results were correlated with histology in 2 of 6 patients (33.3%) (Table 1). For all
patients, genotyping findings agreed with CISH findings.

DISCUSSION

PHM may be underdiagnosed as abortion; it also may be diagnosed as CHM. The diagnosis of CHM is particularly difficult in patients with early evacuated moles, in which the distinct features of villous edema and trophoblastic hyperplasia may not have developed fully. Most moles that are diagnosed initially as PHM, including the HMs in the current study, are diagnosed before 10 weeks. This may explain why the full-blown histologic features of CHM were not conspicuous. It has been reported that in early evacuated CHMs (during the first 5–9 weeks of gestation), in which the distinct features of villous edema and trophoblastic hyperplasia are not fully developed, histologic distinction between CHM and PHM may be particularly difficult. CHM may be misdiagnosed as PHM or even as hydropic abortion.\(^{11–14,32–34}\) PHMs also are quite easily misdiagnosed as CHMs.\(^{30}\) Previous studies demonstrated that most persistent trophoblastic diseases that resulted from histologically diagnosed PHM were in fact CHM according to their diploid DNA patterns.\(^{31}\) Even the presence of amnion or other fetal tissues associated with molar tissue cannot always be considered indicative of a diagnosis of PHM, because these tissues may be androgentic in origin and may indicate the presence of early embryonic development in CHM.\(^{35}\)

Although it is uncommon, PHM also can lead to persistent disease\(^{6,8,9,10,36}\) or even choriocarcinoma.\(^{7}\) In the study conducted by Seckl et al.,\(^{7}\) for example, of the 3000 patients with PHM, only 15 required chemotherapy for persistent disease. The development of metastatic disease in patients with PHM is even more unusual.

Because CHM and PHM genetically are distinct, several techniques that make use of these differences have been developed to help improve the accuracy of diagnosis. Cytogenetic analysis, DNA flow cytometry, and CISH are useful in determining the ploidy of the molar tissue. CHMs usually are diploid, whereas most PHMs are triploid. However, cytogenetic analysis by karyotyping requires fresh tissue for cell culture and preparation of metaphase spreads.

When fresh tissue is not available for karyotyping, ploidy determination can be achieved by DNA flow cytometry and CISH. Both methods can be applied to fixed, paraffin embedded tissues. Using flow cytometry, the large difference of diploid and triploid cell populations, such as in CHM and PHM, can be detected easily.\(^{37–41}\) CISH also aids in the classification of HM by determining ploidy patterns, differentiating between XX and XY CHMs, and deducing the paternal contribution in CHMs using DNA probes specific for the Y chromosome.\(^{25,41,42}\)

PCR aids in the rapid diagnosis and typing of HMs.\(^{43–47}\) Recently, fluorescent-labeled microsatellite primers were used in the genotyping of HMs.\(^{22}\) Using this technique, samples can be run on and analyzed by an automated DNA sequencer, thus enabling quick analyses of large numbers of DNA polymorphisms on different samples at one time.

It is likely that the current study represents the largest series of genetically analyzed metastatic PHMs. We conclude that genotyping using microsatellite analysis and CISH can reliably ascertain the histologic diagnosis and subclassification of HM using formalin-fixed, paraffin embedded tissues of molar villi and decidua, even when maternal tissue is not available for evaluation. Furthermore, this method allows retrospective analysis of archival material. Therefore, this approach is useful in routine surgical pathology reporting. Our findings demonstrate that in some cases, aggressive disease resulting from histologically diagnosed PHM may in fact be CHM. However, a small number of PHMs did progress to metastatic disease. Because it is difficult to distinguish PHM from CHM histologically, all patients with HM should be followed closely.

REFERENCES