Fluorescent In Situ Hybridization
Assessment of Chromosome Copy Number
in Gestational Trophoblastic Disease*

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ABSTRACT

The concurrence of congenital trisomy 8 mosaicism and gestational trophoblastic disease in a forty-two-year-old Gravida IV, Para IV female has been described.¹ In contrast to other cases in the literature, this patient had no additional confounding chromosomal abnormalities other than trisomy 8. To the best of our knowledge, this was the only reported case of constitutional trisomy 8 mosaicism associated with gestational trophoblastic disease, a rare gynecological disease entity in and by itself.

The present report describes fluorescent in situ hybridization (FISH) studies for assessing chromosome 8 copy number on various patient tissues. The results of the FISH studies are compared with each other and with the original cytogenetic studies. It is concluded that the overall frequency of trisomy 8 cells is lower in the FISH studies using archival material than in the original conventional cytogenetic studies. This is true for the uterus and lung tissues with a metastatic tumor. The possible reasons for the somewhat different frequencies found between conventional cytogenetics via GTG-banding and interphase cytogenetics via FISH are discussed.

Introduction

The coincidence of congenital trisomy 8 mosaicism and gestational trophoblastic disease in a 42-year-old woman, which first came to our attention many years ago, has been described.¹²³ Unlike other cases, our patient had no other chromosomal abnormalities other than the trisomy. Our interest in this old and almost forgotten case was rekindled by a recent report in which a hypothesis concerning the meiotic origin of trisomic neoplasms was put forth by Haas and Seyger⁴ (see also Mark⁵).

The patient was a Gravida IV, Para IV female, who had a bilateral partial sal-
pingectomy in August of 1961. In October of 1975 she was admitted to the Rhode Island Hospital where a diagnostic curettage was performed for vaginal bleeding. The histopathological diagnosis at that time was choriocarcinoma. This diagnosis was later revised to placental site trophoblastic tumor. Both choriocarcinoma and placental site trophoblastic tumor are considered to be manifestations of gestational trophoblastic disease. Because the patient had a partial tubal resection, the possibility was raised of de novo choriocarcinoma arising from trophoblastic cells derived from the last pregnancy after a long period of latency. Postmortem samples for tissue culture were subsequently obtained from the metastatic lung tumor and from the skin for cytogenetic studies. It was reasoned at the time that should a Y chromosome be found in the tumor tissue, it would provide compelling evidence for a new, more recent pregnancy.

Cytogenetic studies were performed in skin fibroblasts and lung tumor. To establish unequivocally the presence of mosaicism, it is necessary to study more than one tissue. Although the results of the conventional cytogenetic analysis revealed the presence of a cell line with chromosome 8 trisomy, the patient did not exhibit any phenotypic manifestations usually associated with this mosaicism, such as mild to severe mental deficiency, prominent forehead, deep set eyes, prominent ears, deep palmar creases, etc.

With the advent of recombinant DNA technology and the ability to perform molecular cytogenetic analysis on formalin-fixed, paraffin-embedded archival materials, an interphase cytogenetic study was initiated on other tissues obtained at autopsy, performed some 18 years ago.

Materials and Methods

Hematoxylin and eosin-stained slides from the surgical specimens (approximately 19 years old) obtained after hysterectomy and salpingo-oophorectomy and the autopsy materials (particularly the pulmonary tissue, approximately 18.5 years old) were available for histopathological evaluation. Tissue blocks from both specimens, fixed in 10% formalin and embedded in paraffin, were available for the fluorescent in situ hybridization (FISH) procedure. Selected slides and blocks were chosen for this procedure based on the presence of the tumor cells, which included the primary site (uterus) with adjacent and distant metastases (ovaries and lungs, respectively).

The protocols for conventional cytogenetic studies have been described elsewhere.

For fluorescent in situ hybridization on archival material, modifications of the procedures of Pinkel et al., Mark et al., Mark, Miranda et al., as well as manufacturer’s instructions* were followed. Briefly, formalin-fixed, paraffin-embedded, 4-micron thick tissue sections were applied to silanized slides. The slides were baked at 65°C overnight. Specimens were deparaffinized in xylene and fixed in 100 percent ethanol for 10 minutes each. Pretreatment with 30 percent bisulfide sodium in 2× SSC, pH 7.0 for 60 minutes was followed by 2 minutes of dehydration in a series of 70 percent, 80 percent, 90 percent and 100 percent ethanol solutions. Treatment with proteinase (400 μl of a stock solution, 25 mg/ml, in 40 ml 2× SSC, pH 7.0) for 20 minutes, was followed by dehydration with the same series of ethanol solutions mentioned previously, plus 100 percent acetone for 2 minutes.

The specimens were denatured at 90°C for 12 minutes and left in a humid chamber at 37°C overnight. Specifically, hybridization was performed with a mixture of 2 μl of biotinylated chromosome

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8, α-satellite probe, and 10 μl of Hybrisol VIII per specimen, which was then covered with a glass coverslip, sealed, and prewarmed at 37°C for 30 minutes. The following day the slides were post-washed 2 times in a solution of 4 ml 20x SSC, 16 ml distilled water, and 20 ml formamide (pH 7.0) at 37°C for 15 minutes each, in 0.1x SSC (pH 7.0) for 30 minutes, and then a final wash in 1x PBD (phosphate buffer detergent). For detection, slides were treated with 50 μl of fluorescein-labeled avidin at 37°C for 5 minutes. The interphase nuclei were counterstained with 10 μl of propidium iodide/antifade for each slide. The slides were covered with glass coverslips and examined under a Zeiss epifluorescence photomicroscope using an FITC exciter filter set and Ektachrome ISO 400 color film. Scoring was based on the number of hybridizing signals per cell identified in individual non-overlapping cells as suggested by Kim et al.12 and Hopman et al.13

Results

Pathological findings of the surgical specimens revealed a large fungating mass involving the lower portion of the uterine body measuring 4.8 × 2.5 cm. Histologically, it is a gestational trophoblastic tumor (PSTT) arising in the endometrium and extending into the myometrium.

The post mortem examination showed diffuse pulmonary metastatic choriocarcinoma with urinary bladder implants. There was an intracranial hemorrhage in the posterior portion of the right parieto-occipital region, with a marked internal shift of the midline, tentorium, and brain stem. The cause of this hemorrhage and its relation to the tumor could not be ascertained. Small tumor emboli, if present, may have been difficult to identify. Angioinvasive metastasis seemed likely.

Conventional cytogenetic analyses via GTG-banding (G-banding using trypsin treatment and Giemsa stain) of metaphases derived from both the lung and skin tissues revealed the presence of two clones of cells: an apparently normal clone with a 46,XX karyotype and a clone of hyperdiploid cells with a 47,XX, +8 karyotype. Trisomy 8 was found to be present in 46 percent (16 out of 35) of the cells derived from the lung tumor, whereas 43 percent (13 out of 30) of the cells from the skin tissue culture were trisomic. No mitotic figures were seen in the direct preparation of the tumorous lung nodules. Details have been previously described.1

Interphase cytogenetics via FISH revealed the presence of 19 percent (50 out of 266) cells with three chromosome 8 signals (figure 1) in the lung tumor metastasis. Two other tissues amenable to study were from the uterus and ovary. In normal tissues of the uterus less than 1 percent of the cells were trisomic whereas in tumorous uterine tissues 26 percent of the cells were trisomic. FISH analysis of the ovary yielded 11 percent trisomic signals. The raw data are summarized in table I.

Discussion

The concordance of the karyotypes in the skin and lung tissues first suggested to us that the chromosome 8 trisomy was a constitutional abnormality. The exact proportion of trisomy 8 cells in the various tissues is further defined by the present study. Among the tissues studied in the present project, tumor tissues from the uterus gave the highest frequency of trisomic 8 signals to be followed by tumor tissues from the lung. Tumor tissues derived from the ovary gave the lowest frequency, whereas normal tissues (from the uterus) yielded a background level of near zero. As can be seen from table I, the percentage of trisomic 8 cells
FIGURE 1. Fluorescent in situ hybridization using chromosome 8 specific α-satellite probe on formalin-fixed paraffin-embedded lung tumor tissue.

is lower in the tumor tissues studied by “paraffin FISH” as compared to conventional cytogenetics via GTG-banding. It is difficult to ascertain which of the two techniques reflect more closely the in vivo situation. The FISH percentage may have been lower owing to the age of the specimen. On the other hand, a larger number of cells was sampled in the present interphase cytogenetics study.
TABLE I
Assessment of Chromosome 8 Copy Number in Various Tissues by “Paraffin Fluorescent In Situ Hybridization”

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>1 (Percent)</th>
<th>2 (Percent)</th>
<th>3 (Percent)</th>
<th>4 (Percent)</th>
<th>5 (Percent)</th>
<th>6 (Percent)</th>
<th>Total Studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (tumor)</td>
<td>37 (14)</td>
<td>157 (59)</td>
<td>50 (19)</td>
<td>9 (3)</td>
<td>4 (2)</td>
<td>9 (3)</td>
<td>266</td>
</tr>
<tr>
<td>Uterus (normal)</td>
<td>161 (36)</td>
<td>280 (63)</td>
<td>3 (&lt;1)</td>
<td></td>
<td></td>
<td></td>
<td>444</td>
</tr>
<tr>
<td>Uterus (tumor)</td>
<td>31 (14)</td>
<td>131 (60)</td>
<td>57 (26)</td>
<td>1 (&lt;1)</td>
<td></td>
<td></td>
<td>220</td>
</tr>
<tr>
<td>Ovary (? mixed with mostly normal)</td>
<td>29 (13)</td>
<td>164 (74)</td>
<td>25 (11)</td>
<td>4 (&lt;2)</td>
<td></td>
<td></td>
<td>222</td>
</tr>
</tbody>
</table>

than in the metaphase studies, as metaphases can only be obtained from dividing cells. As discussed previously, the presence of the trisomy 8 cell line in this patient led us to raise the provocative question of its etiologic relation to the tumor. The possibility of the constitutional chromosome 8 trisomy playing a causative role in the development of gestational trophoblastic disease is intriguing. Further discussion, however, is beyond the scope of the present paper.

Acknowledgments

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