Numerical Chromosome Aberrations in a Recurrent Malignant Fibrous Histiocytoma of the Retroperitoneum

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Malignant fibrous histiocytoma (MFH) of the retroperitoneum is relatively rare in Japan. MFHs often recur even if the tumor is resected completely. We describe a case with recurrent MFH of the retroperitoneum. A 49-year-old male was admitted to our hospital for a palpable mass in the right lower abdomen. Ultrasonography and computed tomography demonstrated a solid mass with cystic compartment in the lower pole of the right kidney. The tumor was resected completely, and histological examination showed it was MFH, storiform-pleomorphic type. Thirty-three months later, a local recurrence developed and a second operation was performed. The resected tumor was 4.5×3.9×3.1 cm in size and histological diagnosis was recurrent MFH. We also examined the cytological characteristics of the tumor, using DNA flow cytometric quantification and fluorescence in situ hybridization (FISH) with a set of 14 chromosome-specific DNA probes. DNA contents showed a DNA diploid pattern, however, FISH analysis showed various aberrations of chromosome number such as +1, +2, +7, +8, −10, +11, +12, −16, −17, −18, and +20. These results suggested that chromosomal aberrations may reflect a higher biologic aggressiveness of recurrent MFH.

Key words: malignant fibrous histiocytoma of the retroperitoneum, numerical chromosome aberration, fluorescence in situ hybridization

Introduction

Malignant fibrous histiocytoma (MFH) of soft tissue is one of the most common sarcomas in adults, with a peak incidence in the fifth and sixth decades of life. Most MFHs develop in the lower extremities, gluteal region, shoulder, or the upper extremities. Development of a primary MFH retroperitoneally or intraperitoneally is relatively rare at least in Japan. Since its earliest description, MFH has remained a controversial diagnosis because of the uncertain histogenesis. In addition, there are several grades of malignancies, and MFHs have a high local recurrence rate and a high tendency for dissemination to distant sites. The lung and lymph nodes are the most frequent sites of metastasis. Local recurrence often appears even if the tumor is completely resected. Furthermore, chemotherapies and radiotherapies are not so effective in MFHs. However, to date there are no specific markers for recurrence of MFH.

In the present study, we report a patient with recurrent MFH of the retroperitoneum. We also investigated the cytological characteristics of the tumor by DNA flow cytometry and fluorescence in situ hybridization (FISH) with a set of fourteen chromosome-specific DNA probes. Although DNA contents showed a DNA diploid pattern, FISH results showed various aberrations of chromosome numbers. These findings may correlate with a high biologic aggressiveness of recurrent MFH.

Case Report

A 49-year-old male was admitted to our hospital for further investigation of a palpable mass in the right lower abdomen. The patient complained of chronic
pain in the right lower quadrant of the abdomen that appeared for the first time in March 1990. The past history and family history were unremarkable. On examination, the patient was 163 cm tall and weighed 61.5 kg. There was no lymphadenopathy in the neck, axilla, or inguinal region. Although the abdomen was flat and soft, a rubbery hard and tender mass, 9 cm in diameter, was palpable in the right lower abdomen. Blood chemistry profile was normal except for an increase in serum fibrinogen (580.4 mg/dl) and alanine aminotransferase (47 IU/l). Tumor markers such as the CEA and the CA-19-9 were all within normal limits. Ultrasonography showed a solid mass with cystic compartment, 8 cm in diameter, in the lower pole of the right kidney. Computed tomography (CT) showed a low density mass which was enhanced heterogeneously after injection of the contrast medium.

With a provisional diagnosis of retroperitoneal tumor, laparotomy was performed on June 12, 1990. There was no peritoneal dissemination or liver metastasis. The tumor was located at the lower pole of the right kidney and was well encapsulated. A careful examination during surgery showed no involvement of neighboring organs and the tumor was resected completely. Macroscopically, the tumor (10.0×7.5×4.5 cm) contained a hemorrhagic area and central necrosis. Histologically, the specimen showed proliferation of spindle-shaped tumor cells forming striform pattern. Pleomorphic cells with large nuclei, and multinucleated cells were also identified. Immunohistochemistry by the indirect peroxidase method with antibodies against lysozyme, s-100 protein, desmin, and factor XIIIa was negative, but cells positive for α1-antichymotripsin and α1-antitripsin were found. The final diagnosis was MFH, storiform-pleomorphic type.

The postoperative course was satisfactory and the patient discharged on July 28, 1990. In spite of lack of symptoms, normal blood chemistry, and negative tumor markers, abdominal CT showed a low density mass at the site of the primary lesion on December 22, 1992 on a routine check-up (Figure 1). Because the tumor increased gradually in size, a second operation was performed on April 19, 1993. The resected retroperitoneal tumor was 4.5×3.9×3.1 cm in size and histological diagnosis was a recurrent MFH.

**Nuclear DNA contents and interphase cytogenetics**

The fresh tissue material of the second operation was minced, suspended in 0.2% Triton X-100/PBS(-), filtered through a nylon mesh (#330), and stained with 50 μg/ml propidium iodide (Sigma, St. Louis, MO). The nuclear DNA content was determined by a FACScan (Becton Dickinson, San Jose, CA). The DNA histogram showed a DNA diploid pattern (DNA index=1.00), and the proliferative index (S+G2M/G, +S+G2M) was 12.9% (Figure 2).

**FISH studies** were performed on touch smear from surgical specimens as described previously by our laboratory. Briefly, the slides were incubated with 0.01% pepsin (Sigma, St. Louis, MO)/0.2N HCl for 10 min at 37°C. After washing and dehydration, the slides were denatured for 2 min at 70°C in 70% formamide. Cloned centromeric α satellite DNA from chromosomes 1, 2, 3, 7, 8, 10, 11, 12, 16, 17, 18, 20, X, and Y were used for probes (D1Z5, D2Z, D3Z1, D7Z1, D8Z1, D10Z1, D11Z1, D12Z3, D16Z2, D17Z1, D18Z1, D20Z1, DXZ1, and DYZ3, respectively; Oncor, Gaithersburg, MD). These probes were denatured for 10 min at 70°C in the hybridization mixture, containing 0.5 μg/ml DNA.

**Figure 1.** Computed tomographic scan of the abdomen revealed a low density mass that compressed the right psoas muscle.

**Figure 2.** DNA histogram showed a DNA diploid pattern, and the proliferative index was 12.9%.
probe, 10% dextran sulphate, 2×SSC (sodium saline citrate: 0.3M NaCl, 30mM sodium citrate, pH7.0), and 500 µg/ml herring sperm DNA in 50% formamide. After overnight hybridization at 37°C, the slides were washed with 60% formamide/2×SSC for 10 min at 45°C then incubated with 5 µg/ml fluorescein isothiocyanate avidin DCS (Vector Labs, Burlingame, CA) for 30 min at 37°C. After washing with 0.1% Tween 20 in 4×SSC, the nuclei were counterstained with 1 µg/ml propidium iodide. The number of hybridization signals was counted in 100-200 interphase nuclei in each slide using fluorescence microscopy (BH-2, Olympus). The presence of chromosome aberration exceeding a certain cutoff value was considered to reflect the presence of monosomy, trisomy or tetrasomy (cutoff value: 20%, 15% and 7%, respectively), as described previously. FISH results showed various numerical aberrations such as +1, +2, +7, +8, -10, +11, +12, -16, -17, -18, and +20 (Figure 3, 4 and Table 1).

Table 1. Fluorescence in situ hybridization (FISH) with a set of 14 chromosome-specific DNA probes. FISH results showed various aberrations such as +1, +2, +7, +8, -10, +11, +12, -16, -17, -18, and +20.

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<tr>
<th>Chromosome (DNA probe)</th>
<th>Copy number/Nucleus</th>
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<td>#1</td>
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<td>Y</td>
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Discussion

MFH of soft tissues is one of the most common sarcomas in adults, however, development of a primary MFH of the retroperitoneum is relatively rare in Japan. MFH often recurs even if the tumor is extirpated completely. In our patient, a local recurrence developed 33 months later, but this was not associated with symptoms or abnormal blood tests including tumor markers. The diagnosis of the recurrence was made coincidentally through a routine follow-up CT scanning, thus emphasizing the importance of CT scanning or ultrasonography in the detection of local recurrence. Appropriate surveillance, with regard to the timing and frequency, must be taken into consideration against the risk of recurrence in each individual case. Based on the above diagnostic strategy, one should also evaluate the factors that determine the risk of recurrence.

Nuclear DNA content may be a useful predictor for a high risk of recurrence. The DNA content of nuclei of tumor cells is prognostically relevant in a variety of solid tumors such as lung adenocarcinoma, breast carcinoma, esophageal squamous cell carcinoma, gastric cancer, colorectal cancer, pancreatic carcinoma, and endometrial cancer. Becker et al. examined the

Figure 3. Fluorescence microscopic findings of fluorescence in situ hybridization (×100, objective). A, Nuclei of disomy 3; B, Nuclei of monosomy 17.
prognostic value of DNA analysis by flow cytometry in MFH of the extremities. In the present case, however, the nuclear DNA content showed a DNA diploid pattern. Since changes in the DNA content of less than 4% are not detected by flow cytometry, we examined the numerical chromosome aberration by a more sensitive method (FISH) using a set of 14 chromosome-specific DNA probes.

FISH is a powerful tool for cytogenetic analysis because of its applicability for interphase nuclei. From comparison between interphase and metaphase studies, it became clear that the number of copies of specific metaphase chromosomes could be evaluated by FISH with pericentromeric probes to interphase cells. Aoki et al. compared aberrations of chromosomes 12 and 16 by FISH in 5 cases of myxoid MFHs with those of 9 cases of myxoid liposarcomas. They detected several numeric aberrations of chromosomes 12 and 16 in MFHs compared with fewer aberrations in liposarcomas. In the present case, FISH results showed numerous numerical aberrations such as +1, +2, +7, +8, −10, +11, +12, −16, −17, −18, and +20.

Only a few studies have used conventional cytogenetic analyses of MFH. And only Mandahl et al. analyzed a large series of cases. The karyotypes were characterized by a variety of numerical and structural changes, including ring chromosomes, dicentrics, telomeric associations, trisomy of chromosome 7, 19p+ marker, and double minutes. Rydholm et al. reported that a nonrandom 19p+ maker chromosome appeared in a high percentage of cases and was associated with an increased relapse rate. However, there are no studies describing the relationship between numerical chromosome aberration and prognosis of MFH. In patients with diffuse malignant pleural mesothelioma, Tiainen et al. reported a correlation between mean hyperdiploid and modal chromosome number and shorter survival time. A careful surveillance was necessary in our present patient due to the large number of aberrations of chromosome number.

References