Renal leiomyoma: An immunohistochemical, ultrastructural and comparative genomic hybridization study


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Summary. Renal leiomyoma is a rare neoplasm. We report such a case in a 57-year-old Japanese woman who was found to have a mass in the left kidney. The histological examination disclosed the proliferation of spindle cells showing a benign appearance. Entrapped tubular cells were observed in the peripheral area of the tumor. The immunohistochemical examination of spindle neoplastic cells showed a positive reaction for alpha smooth muscle actin, h-caldesmon, l-caldesmon, calponin, muscle actin, myosin and desmin. Additionally, the ultrastructural examination of the tumor showed membrane caveolae and myofilaments in the cytoplasm. This tumor was considered to show a differentiation into smooth muscle cells. The comparative genomic hybridization of the tumor detected the combined losses of chromosomes 4, 6, 12 and 14 which has not been previously described in renal tumors. Finally, the immunohistochemical panel of smooth muscle markers and ultrastructural and genetic study may be useful in diagnosing renal leiomyoma.

Key words: Renal leiomyoma, Angiomyolipoma, Immunohistochemistry, Ultrastructure, Comparative genomic hybridization

Introduction

Renal leiomyomas are rare neoplasms which occupy 1.5% of the benign lesions and 0.29% of all treated renal tumors (Steiner et al., 1990; Inoue et al., 2000; Romero et al., 2005; Lee et al., 2006). However, there are only two cases with renal leiomyomas with karyotype analysis to date and no reports on renal leiomyomas with comparative genomic hybridization (CGH) analysis (Hisaoka et al., 2002). Therefore, we report a case of renal leiomyoma with a focus on immunohistochemical, ultrastructural and CGH analysis.

Materials and methods

A 57-year-old Japanese woman was found to have a mass in the left kidney. Subsequently, she underwent nephrectomy, but received no postoperative chemotherapy. Convalescence was uneventful two months after the operation.

Specimens obtained from nephrectomy were fixed in 10% buffered formalin and embedded in paraffin, and sections were stained with hematoxylin-eosin. Three µM-thick sections were cut and immunohistochemical staining was performed using a Histofine Simple stain-MAX-PO (multi) kit (Nichirei, Tokyo, Japan). Appropriate tissues were used for positive control of primary antibodies.

Small sections retrieved from formaldehyde-fixed tumor tissue were fixed with 2.5% glutaraldehyde and postfixed with 0.8% osmium tetroxide in phosphate buffer for 1 hour at room temperature. After dehydration in graded ethanol, they were embedded in Epon 812. The ultrathin sections were cut with a Reichert microtome, stained with uranyl acetate and lead citrate, and examined with an electron microscope (JEM-100S; JEOL Ltd, Tokyo, Japan).

CGH analysis was performed as previously described (Kasahara et al., 2005). The tumor area of paraffin-embedded block was removed with a scalpel for DNA extraction. Genomic DNA from the tumor specimens and peripheral blood lymphocytes from
karyotypically normal male controls was isolated by
standard techniques. Reference and tumor DNAs were
labeled by nick translation with Rhodamine-dUTP
(Amersham Pharmacia Biotech, USA) and fluorescein-
dUPT (NEN Life Science Products, Boston, MA),
respectively. The hybridization mixture was composed
of 200ng of tumor DNA, 200ng of reference DNA, and
20ng of Cot-1 DNA (Roche Diagnostics Corporation,
Indianapolis, IN) in 8 µl of hybridization solution, H-
7782 (Sigma-Aldrich Co., St. Louis, MO). The probe
mixture was hybridized to normal male metaphase
spreads (46, XY) for 3 days at 37°C. These hybridized
slides were postwashed in postwash solution (50%
formamide/2xSSC) for 20 minutes at 43°C and washed
twice in 2xSSC for 4 minutes at 37°C and once in
1xPBD (4xSSC/0.05% Tween20) at room temperature.
After three 2-minute washes in 1xPBD, slides were
mounted with a Vectashield (Vector Laboratories,
Burlingame, CA). CGH analysis was performed using an
Olympus BX-50 fluorescence microscope equipped with
single band-pass filters for fluorescein, rhodamine, and
DAPI and with a cooled CCD camera (KAF 1400;
Photometrics, USA) and the Mac Probe version 3.4
analysis system (Applied Imaging Corporation, USA;
Sekitechnotoron, Japan). Nine to ten metaphases were
combined to obtain profiles of the mean ratio and
standard deviation. Chromosomal regions where the
green to red ratio exceeded 1.15 were considered over-
represented (gains), whereas regions where the ratio was
below 0.85 were considered under-represented (losses).

Results

Macroscopic findings

The tumor which measured 7.0x4.0x4.0 cm was
observed in the middle portion of the left kidney and
located in the area extending from renal cortex into renal
capsule. The cut surface of the tumor was whitish
without necrosis or hemorrhage. The tumor showed the
infiltrative margin. Small cysts were focally seen (Fig. 1).

Microscopic findings

Histologically, the tumor was predominantly
composed of spindle cells (Fig. 2a). Nuclear palisading
resembling schwannoma was focally observed (Fig. 2b).
The vascular component was also relatively abundant in
the background. Mitotic figures were less than 2 per 50
high power fields. Hypercellularity, significant
pleomorphism, abnormal mitotic figures or necroses
were completely absent (Fig. 2c). At the periphery of the
tumor, scattered tubular cells without cytological atypia

Table 1. Antibodies employed in the present tumor.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Dilution</th>
<th>Source</th>
<th>Pre-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha smooth muscle actin</td>
<td>1A4</td>
<td>1:100</td>
<td>DakoCytomation, Glostrup, Denmark</td>
<td>heat</td>
</tr>
<tr>
<td>h-caldesmon</td>
<td>h-CD</td>
<td>1:100</td>
<td>DakoCytomation, Glostrup, Denmark</td>
<td>heat</td>
</tr>
<tr>
<td>I-caldesmon</td>
<td>8</td>
<td>1:50</td>
<td>Transduction Laboratories, KY, USA</td>
<td>microwave</td>
</tr>
<tr>
<td>Calponin</td>
<td>CALP</td>
<td>1:50</td>
<td>DakoCytomation, CA, USA</td>
<td>-</td>
</tr>
<tr>
<td>Muscle actin</td>
<td>HHF35</td>
<td>1:50</td>
<td>Enzo Diagnostics, NY, USA</td>
<td>microwave</td>
</tr>
<tr>
<td>Myosin (Smooth)</td>
<td>hSM-V</td>
<td>1:200</td>
<td>Sigma, MO, USA</td>
<td>microwave</td>
</tr>
<tr>
<td>Desmin</td>
<td>D33</td>
<td>1:100</td>
<td>DakoCytomation, Glostrup, Denmark</td>
<td>heat</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>1D5</td>
<td>1:2</td>
<td>DakoCytomation, Glostrup, Denmark</td>
<td>microwave</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>Prf636</td>
<td>1:8</td>
<td>DakoCytomation, Glostrup, Denmark</td>
<td>microwave</td>
</tr>
<tr>
<td>CD117 (c-kit)</td>
<td>polyclonal</td>
<td>1:100</td>
<td>DakoCytomation, CA, USA</td>
<td>heat</td>
</tr>
<tr>
<td>CD34</td>
<td>My10</td>
<td>1:20</td>
<td>BECKTON DICKINSON, CA, USA</td>
<td>heat</td>
</tr>
<tr>
<td>CD35</td>
<td>Ber-MAC-PRC</td>
<td>1:20</td>
<td>DakoCytomation, Glostrup, Denmark</td>
<td>pronounce</td>
</tr>
<tr>
<td>Human Melanosome</td>
<td>HMB45</td>
<td>PD</td>
<td>DakoCytomation, CA, USA</td>
<td>heat</td>
</tr>
<tr>
<td>S-100</td>
<td>polyclonal</td>
<td>1:400</td>
<td>DakoCytomation, Glostrup, Denmark</td>
<td>heat</td>
</tr>
<tr>
<td>CD10</td>
<td>56C6</td>
<td>PD</td>
<td>Novocastra Laboratories Ltd, Newcastle, UK</td>
<td>heat</td>
</tr>
<tr>
<td>Thyroid transcription factor-1</td>
<td>8G7G3/1</td>
<td>1:100</td>
<td>LAB VISION, CA, USA</td>
<td>heat</td>
</tr>
<tr>
<td>Nestin</td>
<td>401</td>
<td>1:50</td>
<td>CHEMICON, CA, USA</td>
<td>microwave</td>
</tr>
</tbody>
</table>
were present.

**Immunohistochemical findings**

Immunohistochemical results are summarized in Table 2. Spindle neoplastic cells as well as normal smooth muscle cells were positive for alpha smooth muscle actin, h-caldesmon, l-caldesmon, calponin, muscle actin, myosin and desmin. However, the spindle cells were negative for estrogen receptor, progesterone receptor, c-kit, CD34, CD35, HMB45, S-100 and nestin. The tubular cells in the peripheral area were negative for CD10 and TTF-1.

**Ultrastructural findings**

Ultrastructurally, the cytoplasm of spindle neoplastic

![Fig. 2. Microscopic findings of the tumor. A. Spindle cells without cytologic atypia proliferate in the renal parenchyma (x 20). B. The pattern of nuclear palisading (arrow) reminiscent of schwannoma is identified (x 40). C. No findings, including hypercellularity, pleomorphism or abnormal mitotic figures are observed anywhere (x 100). D. Glandular structures lacking cytological atypia (arrow) are seen at the peripheral area of the tumour (x 20).](image-url)
cells contained pinocytotic vesicles, namely membrane caveolae, as well as peripheral myofilaments (Fig. 3).

**Comparative genomic hybridization**

Genomic DNA analysis of the tumor specimen by CGH revealed combined losses of chromosomes 4, 6, 12 and 14 (Fig. 4). However, there were no gains of chromosomes.

**Discussion**

Renal leiomyomas are unusual neoplasms which account for 0.29% of all treated renal tumors (Steiner et al., 1990; Inoue et al., 2000; Romero et al., 2005; Lee et al., 2006). They occur more commonly in women than in men. On imaging analysis, tumors form solid, mixed solid and cystic or pure cystic lesion (Tamboli et al., 2000). Macroscopically, tumors are generally located in capsular, subcapsular or peripelvic sites and renal capsule, muscularis of renal pelvis and cortical vascular smooth muscle has been suggested as the origin (Bonsib, 2004; Kunimatsu et al., 2004). It is very important to differentiate renal leiomyomas from angiomyolipoma, schwannoma, stromal-predominant mixed epithelial and stromal tumor and inflammatory myofibroblastic tumor. Angiomyolipomas frequently contain the component of abnormal vessels and adipocytes, and are basically positive for HMB-45 (Bonsib, 1996). It is known that renal capsular leiomyomas may frequently show the positive reactivity for HMB-45 (Tamboli et al., 2000; Bonsib, 2004). In schwannoma, neoplastic cells are generally positive for S-100. In stromal-predominant mixed epithelial and stromal tumor, neoplastic cells are generally positive for estrogen receptor and progesterone receptor (Parikh et al., 2005). In inflammatory myofibroblastic tumor, neoplastic cells are generally negative for h-caldesmon.

**Table 3.** Comparison of abnormalities by CGH between the present tumor and the other renal tumors.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Gain</th>
<th>Loss</th>
<th>Reporter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear cell renal cell carcinoma</td>
<td>5/5q</td>
<td>3p, 8p, 9, 14, 18</td>
<td>Junker et al.</td>
</tr>
<tr>
<td>Papillary renal cell carcinoma</td>
<td>7, 17, 16, 3, 12</td>
<td>1, 2, 6, 10, 13, 17, 21</td>
<td>Junker et al.</td>
</tr>
<tr>
<td>Chromophobe renal cell carcinoma</td>
<td>16, 20</td>
<td>1, 2, 9, 11, 18</td>
<td>Verdorfer et al.</td>
</tr>
<tr>
<td>Carcinoma of the collecting ducts of Bellini</td>
<td></td>
<td>1/1p, 14</td>
<td>Junker et al.</td>
</tr>
<tr>
<td>Oncocytoma</td>
<td></td>
<td>1, 4, 6, 8, 9, 13, 14, 15, 22</td>
<td>Rakoz et al.</td>
</tr>
<tr>
<td>Mucinous tubular and spindle cell carcinoma</td>
<td></td>
<td>9, X, 11q</td>
<td>Brandal et al.</td>
</tr>
<tr>
<td>Juxtaglomerular cell tumor</td>
<td>10</td>
<td>4, 6, 12, 14</td>
<td>Kuroda et al.</td>
</tr>
<tr>
<td>Leiomyoma (present case)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Ultrastructural findings of neoplastic cells. The cytoplasm contains myofilaments (arrowhead) and membrane caveolae are observed at the peripheral area of the cytoplasm (arrow). x 8,000
and the cytoplasm of neoplastic cells ultrastructurally contains no membrane caveolae (Eyden, 2001). The diagnosis of many reported cases of renal leiomyoma has been confirmed by the immunohistochemical application of ASMA and desmin (Wang et al., 2004; Romero et al., 2005). We have previously reported that smooth muscle markers including h-caldesmon, l-caldesmon, calponin and muscle actin express in the human developing renal vascular structures (Naruse et al., 2000). It is well known that myofibroblasts may appear in the stroma of various types of chronic glomerulonephritis or renal cell carcinoma and inflammatory myofibroblastic tumor (Ceballos et al., 2000; Zeisberg et al., 2001; Shimasaki et al., 2005). Myofibroblasts generally do not express h-caldesmon (Ueki et al., 1987; Ceballos et al., 2000). As shown in the present study, we believe that the immunohistochemical panel of smooth muscle markers including h-caldesmon, l-caldesmon, calponin, muscle actin and myosin may be also useful in determining the accurate differentiating direction. Thus, we finally diagnosed this tumor as renal leiomyoma based on immunohistochemistry supported by ultrastructural findings.

Using CGH methods, we found multiple losses of chromosomes. The comparison of chromosomal abnormalities by CGH analysis between the present tumor and other renal tumors is summarized in Table 3. The present tumor seems to be a different in the genetic aspect because the combination of chromosomal abnormalities has not been previously described (Verdorfer et al., 1998; Rakocy et al., 2002; Junker et al., 2003; Brandal et al., 2005). Additionally, the chromosomal abnormality of renal leiomyoma seems to be evidently different from frequent 5q deletion of renal angiomyolipoma that is histologically important in the differential diagnosis (Kattar et al., 1999). Numerical chromosomal abnormalities are frequently observed in renal mesenchymal tumors, especially in chromosome 11 (Guschmann et al., 2002). However, the renal leiomyoma in the present case showed no abnormalities of chromosome 11. The chromosomal abnormality of renal leiomyoma seems to be also different from that of other anatomic sites including uterus and digestive tracts (Sarlomo-Rikala et al., 1998; Levy et al., 2000). CGH studies with more cases of renal leiomyoma will be required in order to confirm that combined chromosomal losses are a universal phenomenon in renal leiomyoma.

In summary, we reported a case of renal leiomyoma in a 57-year-old Japanese woman. The combination studies of immunohistochemistry, electron microscopy and comparative gemonic hybridization appear to be helpful in diagnosing renal leiomyoma.

References


Fig. 4. Results of comparative genomic hybridization of renal leiomyoma. Combined losses of chromosomes 4 (small arrowheads), 6 (large arrowheads), 12 (large arrows) and 14 (small arrows) are observed. There are no gains of chromosomes.
Renal leiomyoma


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