Case Report

Spontaneous Nephroblastoma with Lung Metastasis in a Rat

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Abstract: This report describes a spontaneous nephroblastoma with lung metastasis in a 10-week-old male Crl:CD(SD) rat. Macroscopically, a white mass in the kidney and two white masses in the lung were observed. Histopathologically, the renal mass was located in the cortex of a kidney, and it caused pressure on the surrounding renal parenchyma. Three components could be distinguished in the tumor: blastemal, epithelial (primitive glomerular/tubular structures) and mesenchymal (neoplastic connective tissues) elements. Immunohistochemically, the tumor cells were positive for Wilms tumor 1 protein (WT1) and vimentin. Metastasis was found in the lung. Thus, the case was diagnosed as a nephroblastoma with lung metastasis. (DOI: 10.1293/tox.2013-0059; J Toxicol Pathol 2014; 27: 91–95)

Key words: nephroblastoma, metastasis, immunohistochemistry, rat, spontaneous

Nephroblastoma is uncommon among spontaneous tumors in rats. Furthermore, metastasis of this tumor is extremely rare in rats. The incidence of nephroblastoma has been reported to be around 0.1% or less. Nephroblastoma occurs in both male and female rats and is considered to represent malignant neoplasms1-6. It is known that nephroblastoma has been induced chemically in rats by direct-acting alkylating agents such as N-ethyl-N-nitrosourea (ENU) or N-methyl-N-nitrosourea (MNU)7, 8. In this paper, we report a case of nephroblastoma with lung metastasis that spontaneously occurred in a rat.

The animal was a 10-week-old male Crl:CD(SD) rat obtained from Charles River Laboratories Japan, Inc. (Hino, Gamou, Shiga, Japan), for a toxicity study. The animal protocol was reviewed and approved by the Institutional Animal Care and Ethics Committee of Otsuka Pharmaceutical Co., Ltd. This animal had been healthy, and the masses were found only at autopsy. Grossly, there was one white mass in a kidney (14 mm × 14 mm) and two white masses (8 mm × 8 mm and 2 mm × 2 mm) in a lobe of a lung. No other gross findings were observed. The kidney and lung tissues including the masses were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at a thickness of 4 μm and stained with hematoxylin and eosin (H.E.), periodic acid-Schiff (PAS), Masson’s trichrome and Watanabe’s method for reticulum. In immunohistochemical analysis, sequential sections of masses were labeled with antibodies to Wilms tumor 1 protein (WT1) (diluted at 1:500, rabbit polyclonal antibody, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) as a Wilms tumor cell marker, vimentin (diluted at 1:2500, mouse monoclonal antibody V9, Dako, Carpinteria, CA, USA) as a mesenchymal cell marker, cytokeratin (CK) (diluted at 1:500, mouse monoclonal antibody V9, Dako, Carpinteria, CA, USA) as an epithelial cell marker, Dolichos biflorus lectin (DBA) (diluted at 1:50, horsegram, horseradish peroxidase conjugated, EY Laboratories, San Mateo, CA, USA) as a proximal tubule/collecting duct marker, proliferating cell nuclear antigen (PCNA) (diluted at 1:100, mouse monoclonal antibody PC10, Dako) as a proliferating cell marker, α-smooth muscle actin (αSMA) (diluted at 1:1000, mouse monoclonal antibody 1A4, Dako) as a smooth muscle cell marker and aquaporin 2 (AQP2) (diluted at 1:5000, rabbit polyclonal antibody, Abcam, Tokyo, Japan) as a collecting duct marker. Briefly, the sections were deparaffinized, hydrated and blocked for endogenous peroxidase. Immunohistochemical staining was performed according to polymer-immuno complex method using an EnVision kit (Dako), or the labeled streptavidin-biotin method using an LSAB kit (Dako). Lectin staining was identified by using the direct method. Additionally, the pulmonary tissue was examined immunohistochemically for thyroid transcription factor-1 (TTF-1) (diluted at 1:200, mouse monoclonal antibody 8G7G3/1, Dako) as a type II alveolar cell and Clara cell marker. The other tissues were not examined histopathologically.

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ascending grades of alcohol and embedded in epoxy resin. Ultrathin sections were stained with lead citrate and examined using a JEM 1200 transmission electron microscope (JEOL Ltd., Tokyo, Japan).

Histopathologically, the renal mass was located in the cortex of a kidney, and it caused pressure on the surrounding renal parenchyma. The boundary between the mass and renal parenchyma was well circumscribed. No fibrous capsule had formed, and tumor cells proliferated invasively. In some areas, displaced necrosis and fresh hemorrhages were observed. Three components could be distinguished in the tumor: blastemal, epithelial and mesenchymal elements. Blastemal cells with poorly defined basophilic cytoplasm and large oval nuclei made up a large portion of the components and formed nests (Fig. 1a, b). Additionally, the nests of blastemal cells were separated by neoplastic connective tissues which contained loose spindle-shaped cells (Fig. 1c). Mesenchymal components were stained blue by Masson’s trichrome stain. Epithelial elements had primitive glomerular/tubular structures (Fig. 1d, e). In the nests of blastemal cells, the duct-like structures of monolayer cuboidal or columnar epithelium, with abundant pale cytoplasm and elongated oval nuclei, were surrounded by blastemal cells (Fig. 1f). Mitotic figures were frequently observed among blastemal cells. Discontinuous basement membranes were detected in the primitive tubular structures and duct-like structures by PAS and Watanabe’s method for reticulum staining.

The pulmonary masses were widely demarcated and compressed the surrounding pulmonary parenchyma (Fig. 3a). The masses mainly consisted of nests of blastemal cells and resembled those in the renal mass. The nests were frequently surrounded by a monolayer of cuboidal cells (Fig. 3b). Continuous basement membranes were observed in a monolayer of cuboidal cells by PAS and Watanabe’s method for reticulum staining.

As for the results of immunohistochemical analysis of the renal and pulmonary masses (Table 1 and Table 2), blastemal, epithelial and mesenchymal cells were diffusely positive for WT1 (Fig. 2a-c). Expression of vimentin was diffusely seen in blastemal and mesenchymal cells. Epithelial cells were negative for vimentin (Fig. 2d–f). No signals for CK, DBA and AQP2 were seen in the blastemal, epithelial and mesenchymal components (Fig. 2g–l). Blastemal, epithelial and mesenchymal cells were positive for PCNA. αSMA-positive cells were present in mesenchymal cells; however, no signals were seen in blastemal and epithelial components. In contrast, the duct-like structures in the nests of blastemal cells were negative for WT1 and vimentin but positive for CK and DBA (Fig. 2a, d, g, j). Expression of PCNA was focally detected in the duct-like structures. No duct-like structures were positive for αSMA and AQP2.

In the pulmonary masses, blastemal cells showed similar reactions to those in the renal mass (Fig. 4a, b). They were considered to be identical to the blastemal cells in the renal mass. Monolayer cuboidal cells were exclusively positive for TTF-1 as a type II alveolar cell and Clara cell marker (Fig. 4c, d). No signals for other antibodies were seen in monolayer cuboidal cells.

Ultrastructurally, duct-like structures in the renal masses showed tight junction, desmosome and basement membranes (Fig. 5a). These ultrastructural components were observed in a certain part of the monolayer of cuboidal epithelium in the pulmonary masses (Fig. 5b).

Thus, the tumor was diagnosed as a nephroblastoma.
Fig. 2. Immunohistochemical findings of the renal mass. Blastemal, epithelial and mesenchymal cells reacted diffusely positive for Wilms tumor 1 protein (WT1) (a–c). Expression of vimentin was diffusely seen in blastemal and mesenchymal cells. Epithelial cells were negative for vimentin (d–f). No signals for cytokeratin (CK) and Dolichos biflorus lectin (DBA) were detected in blastemal, epithelial and mesenchymal components (g–l). Duct-like structures in the nests of blastemal cells were negative for WT1 and vimentin but positive for CK and DBA (a, d, g, j). Left column, blastemal cells and duct-like structures; center column, epithelial; right column, mesenchymal. WT1 (a–c). vimentin (d–f). CK (g–i). DBA (j–l). ×500.

Table 1. Immunohistochemical Expression of Each Antigen in Renal Mass

| Primary antibody | Blastemal | Epithelial | Mesenchymal | Duct-like structures |
|------------------|-----------|------------|-------------|---------------------|
| WT1              | ++        | ±          | ±           | –                   |
| Vimentin         | ±         | –          | ±           | –                   |
| CK               | –         | –          | ±           | –                   |
| DBA              | –         | –          | –           | +                   |
| PCNA             | ++        | +          | ±           | +                   |
| αSMA             | –         | –          | –           | –                   |
| AQP2             | –         | –          | –           | –                   |

1) WT1, Wilms tumor 1 protein; CK, cytokeratin; DBA, Dolichos biflorus lectin; αSMA, α-smooth muscle actin; AQP2, aquaporin 2. 2) Expression of PCNA was seen focally in duct-like structures. 3) The intensity grade of immune staining was scored as follows: –, negative; ±, weak; +, moderate; ++, strong.

Table 2. Immunohistochemical Expression of Each Antigen in the Pulmonary Masses

| Primary antibody | Pulmonary masses | Normal lung |
|------------------|------------------|-------------|
| Blastemal        | Monolayer cuboidal cells | Alveolar epithelial cells |
| WT1              | ±                | –           | –           |
| Vimentin         | –                | ±           | –           |
| CK               | –                | –           | ±           |
| TTF-1            | –                | ±           | +           |
| DBA              | –                | –           | –           |
| PCNA             | ++               | –           | –           |
| αSMA             | –                | –           | –           |
| AQP2             | –                | –           | –           |

1) WT1, Wilms tumor 1 protein; CK, cytokeratin; TTF-1, thyroid transcription factor-1; DBA, Dolichos biflorus lectin; αSMA, α-smooth muscle actin; AQP2, aquaporin 2. 2) The intensity grade of immune staining was scored as follows: –, negative; ±, weak; +, moderate; ++, strong.
with lung metastasis based on the above-mentioned characteristics\(^9,10\). Regarding nephroblastoma, the working group on rats and mice of the Japanese Society of Toxicologic Pathology (JSTP)\(^9\) proposed the classification into the blastemal, epithelial and mesenchymal types depending on each dominant cell component. In our case, the majority of tumor components were blastemal cells. Therefore, we classified it as the blastemal type.

Immunohistochemically, the duct-like structures in the renal mass were different from blastemal, mesenchymal and
epithelial cells. Additional examinations might be required to determine whether these structures were tumor components or not. The monolayer cuboidal cells in the pulmonary masses were exclusively positive for TTF-1 as a type II alveolar cell and Clara cell marker. They were considered to derive from alveolar epithelial cells, not from tumor cells. In this report, we described the histological, immunohistochemical and ultrastructural characteristics of a spontaneous nephroblastoma with lung metastasis in a male rat.

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