Case Report

Sebaceous Gland Metaplasia in a Mammary Fibroadenoma Developing in a Female Donryu Rat

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Abstract: A mammary fibroadenoma with prominent sebaceous gland-like structures in a female Donryu rat aged 67 weeks was immunohistochemically investigated. The tumor demonstrated a mixture of both epithelial and connective tissue components, the former having both single- and multi-layered patterns. Characteristic sebaceous gland-like structures were apparent in connection with multi-layered epithelium. Area of single-layered epithelium comprised well-differentiated myoepithelial cells positive for alpha-smooth muscle actin (alpha-SMA), similar to mammary ducts. In contrast, many epithelial cells on the basal sides of multi-layers were negative for alpha-SMA, but positive for cytokeratin 14 (CK14), suggesting pluripotency. From these results, we diagnosed this case as a mammary fibroadenoma with sebaceous gland metaplasia. (J Toxicol Pathol 2002; 15: 73–77)

Key words: fibroadenoma, mammary gland, sebaceous gland metaplasia, spontaneous, Donryu rat

Mammary fibroadenoma is one of the most common spontaneous tumors in aged Sprague-Dawley, Fischer-344, and Donryu rats1-3. It is characterized by proliferation of both epithelial and fibrous connective tissue components with no atypia. As a variant, rat fibroadenomas with squamous epithelium or sebaceous cell-like structures have been previously described4, although they are very rare. Recently, we encountered a mammary fibroadenoma with prominent sebaceous gland-like elements in a female Donryu rat. In this report, we described its morphological characteristics, including histochemical and immunohistochemical features, and discussed the origin of sebaceous gland-like structures.

The animal was one of 39 female rats given a single dose of 200 mg N-propyl-N-nitrosourea (PNU) per kg body weight by stomach tube at 10 weeks of age. The animal was euthanized at 67 weeks of age on becoming moribund. At necropsy, a large subcutaneous mass was found, in addition to an abscess of the lung, hematoma of the uterus, and enlargement of the spleen. Animal care and use followed the NIH Guide for the Care and Use of Laboratory Animals.

All lesions and major organs were fixed in 10% neutral buffered formaldehyde solution and processed routinely for histopathological examination after H-E staining. In addition, deparaffined sections of the subcutaneous mass were processed for Masson’s trichrome histochemistry, and for immunohistochemical analysis using mouse monoclonal antibodies against alpha-smooth muscle actin (alpha-SMA; clone 1A4, DAKO Japan, Kyoto) and cytokeratin 14 (CK14; clone LL002, Novocastra Laboratories Ltd, Newcastle upon Tyne). For this latter, sections were incubated with one of the antibodies overnight at 4°C, and subsequently with secondary antibodies conjugated to peroxidase labeled-dextran polymers (EnVision+, DAKO Japan). Normal sebaceous glands in a female Donryu rat aged 12 weeks were examined immunohistochemically in the same manner for comparison with the present case.

Histologically, the subcutaneous well-circumscribed tumor tissue compressing the adjacent normal tissue was composed of proliferation of both epithelial cells resembling mammary ducts and abundant connective tissue surrounding the epithelial components (Fig. 1a). In the epithelial component, both single- and multi-layered patterns were observed (Fig. 1a), with transitions between the two in part. In the single-layered epithelium, fusiform or stellate myoepithelial cells predominated but they could not been detected in multi-layered areas. There were no cellular atypism and few mitotic figures of epithelial cells.

In the present case, the most striking finding was the presence of sebaceous gland-like structures in association and connecting with multi-layered epithelium (Fig. 1b).
Many of the component cells possessed finely granular to foamy cytoplasm, with basaloid cells having hyperchromatic nuclei and scant cytoplasm at the periphery, resembling their counterparts in normal sebaceous glands.

Histochemical analysis by Masson’s trichrome revealed both epithelial components to be surrounded by large amounts of collagen fibers. The immunohistochemical results are summarized in Table 1. In our case, well-differentiated myoepithelial cells along the single-layered epithelium were generally positive for alpha-SMA (Fig. 2a), but only a few positive cells were detected in the multi-layered areas (Fig. 2b). Cells in the sebaceous gland-like structures as well as normal sebaceous glands in a female Donryu rat were negative for alpha-SMA.

Fig. 1. a) The subcutaneous mass is composed of both epithelial and connective tissue components, the former presenting as ductal structures resembling mammary ducts, with large amounts of surrounding connective tissue. Single-layered (right) and multi-layered (left) epithelia are observed. × 50. H-E. b) Sebaceous gland-like structure observed in association with and connected to a multi-layered area. × 125. H-E.
With CK14, myoepithelial cells in the single-layered epithelium were weakly positive (Fig. 3a). While elements on the basal sides of multi-layered epithelium were strongly positive (Fig. 3b), positive cells were found scattered in the luminal epithelium of both epithelial types (Figs. 3a, b). Cells of the sebaceous gland-like structure as well as the normal sebaceous gland were strongly positive for CK14 (Figs. 4a, b), along with the Malpighian stratum including basal cells in the normal skin (Fig. 4b).

The histopathological results described above demonstrated that the present case should be diagnosed basically as a mammary fibroadenoma. While there were obvious differences from the typical tumor, we could rule out the possibility of a mixed mammary fibroadenoma and sebaceous gland adenoma, because of the clear connections between multi-layered epithelium and the sebaceous gland-like structures.

The present fibroadenoma case was atypical in having both single-layered and multi-layered patterns, the former predominating in most cases in rats. Immunohistochemically, in human and rat mammary glands, myoepithelial cells are positive for alpha-SMA and CK14, and basal epithelial/myoepithelial cells are reported to be positive for CK14 in wide area. **: Almost all cells responsive. ++: Almost all cells responsive in wide area. N: No response to antibodies.

Table 1. Summary of Immunohistochemical Characteristics —Alpha-SMA and CK14—

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Normal human and rat mammary glands*</th>
<th>Pluripotent cells in human mammary glands**</th>
<th>Single-layered epithelium</th>
<th>Multi-layered epithelium</th>
<th>Sebaceous gland-like structure</th>
<th>Normal rat sebaceous glands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Myo.1</td>
<td>Basal2</td>
<td>Myo.</td>
<td>Luminal6</td>
<td>Epithelial cells in the basal side</td>
<td>Epithelial cells in the luminal side</td>
</tr>
<tr>
<td>Alpha-SMA</td>
<td>Y</td>
<td>No data</td>
<td>N</td>
<td>Y</td>
<td>Y (+)</td>
<td>Y (±)</td>
</tr>
<tr>
<td>CK14</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y (±)</td>
<td>Y (±)</td>
</tr>
</tbody>
</table>

*: According to the literatures5–12. **: According to the literature7. No data: No record in the literature to our knowledge. 1: Myoepithelial cells. 2: Basal epithelial cells. 6: Luminal epithelial cells. Y: Specific response to antibodies. ±: A few cells responsive, or almost all cells weakly responsive. +: Almost all cells responsive. ++: Almost all cells responsive in wide area. N: No response to antibodies.

Fig. 2. a) Myoepithelial cells lining the single-layered epithelium are positive for alpha-SMA. Smooth muscle fibers in the blood vessel are also positive (arrows). × 152. Alpha-SMA. b) A few myoepithelial cells along multi-layered epithelium are positive for alpha-SMA (arrow). × 152. Alpha-SMA.

Shimomoto, Yoshida, Takahashi et al.
mammary ducts is considered to contain pluripotent cells capable of developing into either luminal epithelial or myoepithelial cells\textsuperscript{13}. In epitheliosis of human mammary glands, some intraluminal proliferative cells are positive for CK14 and negative for alpha-SMA, and it has been suggested that these are post-stem or intermediate cells which generate the luminal epithelium\textsuperscript{7}. Our results thus indicate that the CK14-positive and alpha-SMA-negative cells on the basal side of multi-layered epithelium might be similarly pluripotent and some of them giving rise to sebaceous gland-like structures. The lack of malignant features, connection with the multi-layered epithelium and similarity to normal sebaceous glands support the conclusion of metaplasia.

In conclusion, we diagnosed this case as a mammary fibroadenoma with sebaceous gland metaplasia. We could not find similar mammary fibroadenomas in other rats of the

Fig. 3. a) Myoepithelial cells lining single-layered epithelium are weakly positive for CK14. Some of the luminal cells are also positive (arrows). \(\times\) 152. CK14. b) On the basal sides of the multi-layered epithelium, large numbers of epithelial cells are positive for CK14. Some of the luminal cells are also positive (arrows). \(\times\) 152. CK14.

Fig. 4. a) Sebaceous gland-like structures partially positive for CK14. \(\times\) 95. CK14. b) Normal sebaceous gland in a female Donryu rat aged 12 weeks. The sebaceous gland is positive for CK14 (arrow) as well as cells of the Malpighian stratum including basal cells in the skin (arrowhead). \(\times\) 251. Left: H-E. Right: CK14.
same group, although a total of 14 lesions were observed in the 39 rats of the group, suggesting that the sebaceous gland-like metaplasia was spontaneous in nature.

References