Case Report

Histopathological and Immunohistochemical Characterization of Spontaneously Occurring Uterine Deciduomas in Young Adult Rats

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Abstract: Uterine deciduomas were found in two female virgin rats, a 15-week-old Lewis rat and a 7-week-old Sprague-Dawley rat. The firm white nodules were located at the base of unilateral uterine horns and were approximately 6 mm and 4 mm in diameter. Histopathologically, the nodules were composed of three areas, each with a distinct type of proliferating cells: large epithelioid decidual cells with round nuclei, prominent nucleoli and abundant eosinophilic cytoplasm (antimesometrial region); compact spindle-shaped cells with oval nuclei and vacuolar cytoplasm (transitional region); and pleomorphic and spiny cells with round to oval nuclei and compact eosinophilic cytoplasm (mesometrial region). These cells proliferated in sheet-like arrangements and transformed into the other types of cells located in surrounding regions. Immunohistochemically, proliferating cells in all regions were strongly positive for proliferating cell nuclear antigen. The proliferating cells were positive for vimentin, and large decidual cells were positive for common acute lymphoblastic leukemia antigen 10, a marker of uterine interstitial cells. Large decidual cells were positive for α-smooth muscle actin and desmin, suggesting differentiation into muscular cells. Progesterone receptor was expressed in all cell types; however, estrogen receptor α was not expressed in the antimesometrial region. These extremely rare tumor-like nodules represent nonneoplastic lesions referred as decidual reactions of endometrial interstitial cells, and their biological behavior is that of a space-occupying benign tumor in young rats. Our cases might provide information as a historical control in toxicity and pharmacological studies in rats. (DOI: 10.1293/tox.26.61; J Toxicol Pathol 2013; 26: 61–66)

Key words: deciduoma, decidual reaction, immunohistochemistry, rat, uterus

Spontaneous primary tumors and tumor-like lesions of the rodent uterus are extremely rare in young rodents. Hyperplastic lesions of decidual stromal cells, so-called decidual reactions/deciduomas, have been found in the uterus or subserosal abdominal locations in rats1-3, mice4,5, guinea pigs,6 rabbits,7 dogs,8 monkeys,9,10 and humans9,10. These lesions are characterized by focally extensive areas of hyperplastic and hypertrophied eosinophilic, round, oval to somewhat elongated cells, which are thought to be endometrial stromal cells. Decidual stromal cells are derived from the fibroblast-like cells within the endometrium, which maintain their progesterone receptors in the presence of progesterone11. This proliferating lesion is often referred to as deciduoma and is thought to be a proliferative response of stromal endometrial cells that is histologically similar to decidual implantation sites2. This change is infrequently seen in young adult rats used in subacute toxicity studies, and the incidence of decidual reactions/deciduoma in young female F344 rats examined in National Toxicity Program studies is 1.3% (2 of 159 rats)12. Only 5 spontaneous cases of decidual reactions/deciduoma were identified in approximately 12000 Sprague-Dawley (SD) rats at 3- to 8-months of age13. In B6C3F1 mice used in National Toxicity Program studies, this change was seen in only one mouse3. Few studies on the immunohistochemical characterization of spontaneously occurring deciduoma have been reported13. We encountered two uterine deciduomas in a young Lewis rat and a SD rat and examined their histopathological and immunohistochemical features in the present study.

The experimental protocol and all animal procedures were approved by the Animal Care and Use Committee of Kansai Medical University and were in accordance with the university’s guidelines for animal experimentation. Case 1 was a 15-week-old female LEW/CrlCrlj (Lewis) rat, and Case 2 was a 7-week-old female Crl:CD (SD) rat. The 2 rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan) and used in different mechanistic studies of chemical-induced toxicity and carcinogenesis. The rats were housed in plastic cages with paper-chip bedding (Paper Clean, SLC, Hamamatsu, Japan) in an air-conditioned room at 22 ± 2°C and 60 ± 10% relative humidity with a 12-h light/dark cycle and fed a commercial diet (CMF 30

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Gy; Oriental Yeast, Chiba, Japan) and tap water *ad libitum*.

The rats were anesthetized with isoflurane (Forane®; Abbott Japan, Tokyo, Japan), sacrificed by exsanguinations via abdominal aortic transection, and subjected to complete necropsies. One small mammary carcinoma was seen in Case 1, but none were seen in Case 2. No abnormalities were observed except in the mammary glands macroscopically. The uterus and other reproductive organs were fixed over

| Primary Antibody | Source | Clone | Dilution | Antigen Retrieval | Detection |
|------------------|--------|-------|----------|-------------------|-----------|
| PCNA             | Leica  | PC10  | 1:100    | 115°C for 10 min  | Proliferating cells |
| Pancytokeratin   | Dako   | AE1/AE3| 1:50     | 115°C for 10 min  | Epithelial cells    |
| Vimentin         | Dako   | V9    | 1:50     | 115°C for 10 min  | Mesenchymal cells   |
| CD10             | Novocastra | 56C6  | 1:100    | 115°C for 10 min  | Uterine interstitial cells |
| SMA              | Dako   | 1A4   | 1:50     | Not conducted     | Smooth muscular cells |
| Desmin           | Dako   | D33   | 1:50     | Not conducted     | Muscular cells      |
| S-100 (α, β)     | Dako   | Polyclonal | 1:500   | Not conducted     | Schwann cells, adipocytes |
| ER               | Novocastra | 6F11  | 1:40     | 115°C for 10 min  | Estrogen receptor   |
| PgR              | Biodesign | 10A9  | 1:50     | 115°C for 10 min  | Progesterone receptor |

1) ER, estrogen receptor α; PCNA, proliferation cell nuclear antigen; PgR, progesterone receptor; SMA, α-smooth muscle actin.
2) Biodesign (Saco, ME, USA), Dako (Carpinteria, CA, USA), Leica Biosystems (Newcastle upon Tyne, UK), Novocastra (Newcastle upon Tyne, UK). 3) Primary antibodies were incubated with tissue samples for 1 hr at room temperature, with the exception of ER antibody, which was incubated overnight at 4°C. The antigen-antibody complexes were identified by using a streptavidin-biotin (LSAB) staining kit (Dako) according to the manufacturer’s instructions. The reaction products were visualized with 3-3’-diaminobenzidine tetrahydrochloride. 4) Antigen retrieval was conducted by pressure-cooker heating (Pascal, Dako).

A solitary uterine nodule was detected in only one rat of approximately 100 animals used in each study. The white and firm solitary nodules were found in both rats at the base of unilateral uterine horns and were approximately 6 mm and 4 mm in diameter. Histopathologically, these nodules had collapsed the uterine lumens with erosion of the overlying endometrial surface and hemorrhage (Fig. 1a, 1b). The nodules contained 3 areas of different proliferating cell types. Large epithelioid giant cells with round nuclei containing coarsely vesicular chromatin in large oval nuclei with one to four variably shaped nucleoli and abundant eosinophilic and coarsely granular cytoplasm proliferated in the antimesometrial side of the uterus (decidual cells) (Fig. 2a). Occasionally, multinucleate or binucleate cells were seen in this region. A small number of large cells possessed abundant PAS-positive granules in this region (data not shown). The mesometrial region contained pleomorphic and spiny cells with round to oval nuclei and abundant coarse clear cytoplasmic vacuoles (Fig. 2c). Small spindle-shaped cells with oval nuclei and finely vacuolar cytoplasm were located between both regions (transitional region) (Fig. 2b). These cells proliferated in sheet-like arrangements and transformed into the other cell types located in surrounding regions. The cells of the nodules were supported by a fine fibrovascular stroma with small congested blood vessel profiles (Fig. 2c). On the antimesometrial side, there was an expansile, partially encapsulated nodular mass composed of three poorly defined layers: the capsule, the basal zone with residual endometrial glands, and the myometrium (Fig. 2d). No abnormal changes were detected histopathologically in the other uterine horns, vaginas, and bilateral ovaries of these rats (data not shown).

The results of the immunohistochemical analysis are summarized in Table 2. The nuclei of most decidual cells in both rats were strongly positive for PCNA in all three regions (Fig. 3a), showing highly proliferative activity of the nodules. The tumor cells in all regions were also strongly positive for vimentin, and the cytoplasm of large decidual
cells was weakly positive for CD10, showing that the tumor cells originated from uterine interstitial cells (Fig. 3b). The cytoplasm of these cells was also moderately or strongly positive for SMA and desmin, probably suggesting differentiation into muscular cells (Fig. 3c, 3d). ER was expressed weakly to strongly in the nuclei of compact spindle-shaped cells and starry-formed cells in the transitional and mesometrial regions (Fig. 3e); however, no signals were seen in the antimesometrial region. In contrast, PgR was expressed moderately to strongly in the nuclei of all types of decidual cells.
cells in the three regions (Fig. 3f). No proliferative cells were positive for S-100 in any regions. The cells comprising the capsule and myometrium were weakly to moderately positive for vimentin, SMA, desmin, ER, and PgR. Endometrial glands in the basal zone were positive for CK and ER, which was similar to the normal endometrial gland.

Table 2. Immunohistochemical Expression of Antigens in the Nodules

| Primary antibody | Case 1 | Case 2 |
|------------------|--------|--------|
|                  | Antimesometrial region | Transitional region | Mesometrial region | Antimesometrial region | Transitional region | Mesometrial region |
| PCNA             | ++++ 2) | +++ | +++ | +++ | +++ | +++ |
| Pancytokeratin   | –      | –   | –   | –   | –   | –   |
| Vimentin         | +++    | +++ | +++ | +++ | +++ | +++ |
| CD10             | +      | –   | –   | +   | –   | –   |
| SMA              | +      | –   | –   | ++  | –   | –   |
| Desmin           | +      | –   | –   | +++ | –   | –   |
| S-100 (α, β)     | –      | –   | –   | –   | –   | –   |
| ER               | –      | ++  | +++ | –   | +   | +++ |
| PgR              | ++     | ++  | +++ | ++  | ++  | +++ |

1) ER, estrogen receptor α; PCNA, proliferation cell nuclear antigen; PgR, progesterone receptor; SMA, α-smooth muscle actin.  
2) The intensity of staining was scored as negative (–), weak (+), moderate (++) or strong (+++).
CD10, a marker for uterine interstitial cells. Although cells at the antimesometrial region also weakly expressed the decidual tissue during days 12-16 of pseudopregnancy.

In our cases, the proliferative decidual cells showed strong and diffuse expression of vimentin, desmin, SMA, and desmin increases in rat decidual cells in both in vitro and in vivo models, as these proteins are valid markers of stromal cell differentiation. During the implantation stage of the normal rat pregnancy, the localization of vimentin is widespread in decidual cells, while desmin is present densely in decidual cells of the antimesometrial region where implantation occurs. In our cases, the proliferative decidual cells showed strong and diffuse expression of vimentin; however, desmin was expressed in the decidual cells in the antimesometrial region. Similar to the expression of desmin, SMA was expressed in the decidual cells of the antimesometrial region, suggesting myogenic or myofibroblastic differentiation. In our decidualomas, large decidual cells at the antimesometrial region also weakly expressed CD10, a marker for uterine interstitial cells. Although normal endometrial stromal cells are positive for CD10 in humans, these signals were not detected in the normal endometrial stromal cells in rats.

ER and PgR are expressed in the myometrium of the rat during the decidualization process and in spontaneously occurring decidualomas. Progesterone and small amounts of estrogen are required for the sensitization of the uterus before a decidualizing stimulus. Continuous presence of progesterone is essential and adequate for proliferation of the decidual cells and for the growth and maintenance of the decidua.

In our cases, PgR and ER were expressed strongly in the mesometrial region; however, ER was not expressed in the antimesometrial region. According to a previous report, the involvement of both receptors may suggest that as the decidual cells become differentiated, they become enriched in PgR and impoverished in ER.

Although Lewis rats and SD rats are frequently used in pharmacological studies and toxicity studies, respectively, historical control data for uterine decidual lesions in young rats of both strains have not been well reported. In our cases, proliferating decidual cells expressed PCNA, vimentin, CD10, SMA, and desmin. PgR was expressed in all cell types, but ER was not expressed in the antimesometrial region. Although additional immunohistochemical analyses are needed to understand the biology of spontaneously occurring decidualomas, our two cases might provide valuable information as a historical control for Lewis and SD rats.

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