Case Report

Cutaneous Hybrid Cyst in a Sprague-Dawley Rat

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Abstract: This report describes a spontaneous hybrid cyst in a Sprague-Dawley (SD) rat. A 52-week-old, male SD rat had a cutaneous cyst on the left mystacial pad. Histologically, the cyst wall showed infundibular differentiation with keratohyalin granules in the granular layer and matrical differentiation comprising basaloid epithelial cells with trichohyalin granules. The cyst cavity was filled with lamellar, flaky keratin and aggregates of shadow cells. Immunohistochemically, the infundibular-type epithelium was positive for cytokeratin (CK) AE1/AE3, CK KL1 and CK14 but negative for CK15, whereas the matrical-type epithelium was negative for all four CK isoforms examined. These immunohistochemical properties of the infundibular- and matrical-type epithelia were similar to those of the infundibulum and inferior segment of normal hair follicles, respectively. Based on these findings, the cyst was diagnosed as a hybrid cyst, comprising more than one type of cyst arising from various parts of the pilosebaceous unit. (DOI: 10.1293/tox.25.175; J Toxicol Pathol 2012; 25: 175–178)

Key words: kin, hybrid cyst, epidermal inclusion cyst, cytokeratin, Sprague-Dawley rat

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bodies shown in Table 1. Peroxidase-conjugated anti-mouse immunoglobulin G (Histofine Simple Stain MAX-PO(M); Nichirei, Tokyo, Japan) was used as a secondary antibody. After immunoreaction, the sections were colorized with diaminobenzidine and counterstained with Mayer’s hematoxylin. Intact skin samples from five rats (n = 5) were used as controls to compare the immunohistochemical properties of CK expression with hair follicles in normal rats.

Table 1. Immunohistochemistry

| Antibody         | Clone | Dilution | Antigen retrieval | Antibody source  |
|------------------|-------|----------|-------------------|-----------------|
| Cytokeratin AE1/AE3 | 1:50  | trypsin  | Dako              |
| Cytokeratin KL1   | 1:100 | MW       | Immunotech        |
| Cytokeratin 14    | LL002 | MW       | Serotec           |
| Cytokeratin 15    | CBL272| NT       | Millipore         |

* Trypsin = 0.1% trypsin, 37°C, 30 min; MW = microwave/citrate buffer (pH 6.0), 90°C, 10 min; NT = no treatment. * Dako, Glostrup, Denmark; Immunotech, Marseille, France; Serotec, Wiesbaden, Germany; Millipore, Temecula, CA, USA.

Fig. 1. Low-power view of the cutaneous cyst. The cyst develops and extends into the underlying fat. The upper portion opens into the overlying epidermis. HE. Bar = 1 mm.

Fig. 2. The cyst wall is lined with a mixture of the infundibular-type epithelium (right) and matrical-type epithelium (left). The former shows infundibular keratinization with lamellar keratin and keratohyalin granules (arrows), and the latter shows abrupt keratinization forming aggregates of shadow cells (*) and trichohyalin granules (arrow heads). HE. Bar = 200 μm.

Fig. 3. Immunostaining of cytokeratin (CK) in the cyst wall. The lining consists of the infundibular-type epithelium (right) and matrical type epithelium (left). The former is positive for CK AE1/AE3 (A), CK KL1 (B) and CK14 (C) but negative for CK15 (D). The latter is negative for all four CK isoforms examined.
Histologically, a single dermal-based cyst had developed and extended into the underlying fat (Fig. 1). The upper portion of the cyst was open, forming a C-shaped structure that connected with the overlying epidermis. The epithelial lining and keratinization pattern of the cyst wall was divided into two types: infundibular and matrical (Fig. 2).

The former comprised an infundibular-type epithelium with keratohyalin granules in the granular layer resulting in keratinization with laminated corneocytes. The latter, which was adjacent to the infundibular-type, showed a matrical-type epithelium, which was lined by basophilic basaloid epithelial cells with scant cytoplasm and hyperchromatic nuclei, and was abruptly keratinized, forming aggregates of shadow cells. Trichohyalin granules and PAS-positive cells containing glycogen were observed within the matrical-type, indicating differentiation into the inner root sheath. The two types of epithelium in turn formed a line, and the transition was irregularly developed. The cyst cavity was filled with lamellar or flaky keratin in addition to the aggregates of shadow cells, but no hair fragments were observed. Several lymphocytes and neutrophils were observed inside the cyst and the outer layer of the cyst wall.

The results of immunohistochemical examinations of the cyst in this case and normal tissues are summarized in Table 2. The expression of CK isoforms in the cyst wall differed between the types of epithelium lining. The infundibular-type epithelium was positive for CK AE1/AE3, CK KL1, and CK14, but was negative for CK15 (Fig. 3). In contrast, the matrical-type epithelium was negative for all four CK isoforms examined (Fig. 3). The cystic lesion was finally diagnosed as a hybrid cyst on the basis of morphological and immunohistological characterization.

Hybrid cysts comprise more than two structures of the pilosebaceous unit, and the combination of these structures can vary. The following combinations have been described in humans: infundibular cyst-trichilemmal (isthmic) cyst, infundibular cyst-matrical cyst, trichilemmal cyst-matrical cyst, eruptive vellus hair cyst-steatocystoma, trichilemmal cyst-eruptive vellus hair cyst, and infundibular cyst-apocrine hidrocystoma. The most common combination in humans, as well as dogs, is the infundibular cyst-trichilemmal cyst. Histologically, the combination type of the present case was infundibular cyst-matrical cyst, which was also confirmed by immunohistochemical examinations. The immunohistochemical properties of the infundibular- and matrical-type epithelia within the cyst in this case were similar to those of the infundibulum and inferior segment in normal rat hair follicles, respectively (Table 2). This strongly suggests that the epithelial lining of this cyst showed divergent differentiation to an infundibulum and inferior segment of the hair follicle.

The etiology of hybrid cysts is unclear, but they probably originate from follicular stem cells. Rodins et al. suggested that β-catenin, which is an important requirement for follicular stem cells that are involved in hair follicle development, plays a role in the pathogenesis of hybrid cysts. Papilloma virus has also been demonstrated in epidermal inclusion cysts of European harvest mice, which indicates a papillomaviral etiology for this cyst.

McMartin et al. reported that epidermal inclusion cyst can occur spontaneously in 1.7% of male and 0.3% of female SD rats. The frequency of hybrid cysts in rats is unclear, but a certain proportion of hybrid cysts may be diagnosed as epidermal inclusion cysts during routine examinations. Investigation of the combinations of different types of epithelium in this disease is useful to understand the pathogenesis of pilosebaceous-derived tumors in rats, and immunohistochemical examination using CK isoforms could help to clarify the differentiation of the epithelial lining of hybrid cysts.

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