NOTE
Pathology

Pulmonary adenofibroma in a sika deer

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ABSTRACT. A solitary firm nodule was found in the lung of a sika deer (*Cervus nippon yesoensis*). Histologically, it was a biphasic lesion composed of epithelial and stromal cell elements and exhibited a leaf-like growth pattern. The epithelial cells were immunohistochemically positive for pancytokeratin, cytokeratin 7, napsin A, and thyroid transcription factor-1, and the stromal cells were positive for vimentin and partially positive for desmin and α-smooth muscle actin. These observations were consistent with pulmonary adenofibroma, which is an extremely rare lesion in humans. To the best of our knowledge, this is the first reported case of pulmonary adenofibroma in an animal.

KEY WORDS: biphasic, *Cervus nippon yesoensis*, immunohistochemistry, pulmonary adenofibroma, sika deer

Pulmonary adenofibromas are benign lesions affecting the lung parenchyma, and they are characteristically composed of complex epithelial and stromal components [1–6, 8–12]. As for the pathogenesis of pulmonary adenofibromas, it remains unclear whether these lesions are hamartomas or true neoplasms. Pulmonary adenofibromas are extremely rare in humans, and no cases have been reported in the veterinary literature. In this paper, we present a case of pulmonary adenofibroma in a sika deer, which was histologically and immunohistochemically consistent with the reported human cases.

The deer involved in the present case was a female sika deer (*Cervus nippon yesoensis*), which was captured in the eastern district of Hokkaido, the northern island of Japan, and had been farmed until slaughter for about 8 months. The deer weighed 73 kg and was estimated to be ≥3 years old at slaughter. Grossly, two firm nodules, a larger one measuring 2.5 × 2 × 2 cm and a smaller one measuring 1.5 × 1 × 1 cm, were detected in the parenchyma of the right cranial and caudal lobes of the lung, respectively. In an examination of the nodules' cut surfaces, the larger nodule was found to be composed of well-demarcated, tan-pink rubbery tissue (Fig. 1), and the smaller nodule was shown to be composed of pale-tan tissue. There were no significant lesions in the other organs, but liver flukes were found in an intrahepatic bile duct. The pulmonary nodules were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax. Sections were then stained with hematoxylin and eosin.

Histologically, the smaller pulmonary nodule was encapsulated caseous necrosis with intralesional branching hyphae, which was consistent with pulmonary aspergillosis. The larger nodule was composed of a combination of epithelial and stromal components and was well demarcated from the adjacent lung parenchyma by a focal, thin fibrous capsule. The stromal component was larger than the epithelial component and consisted of proliferating spindle-shaped cells arranged in leaf-like and patternless growth patterns (Fig. 2). In the region exhibiting a leaf-like growth pattern, stromal cells had formed multiple, small, club-shaped structures, which projected into the lumen. This resulted in the formation of staghorn-like, branching spaces, which were lined by a single layer of flattened to cuboidal epithelial cells (Fig. 3a and 3b). Large cystic spaces were occasionally seen together with eosinophilic fluid in the lumen. In the region that exhibited patternless growth, spindle-shaped cells proliferated haphazardly, and isolated, small, gland-like spaces, which were lined by a single layer of flattened to cuboidal epithelial cells, were scattered (Fig. 4a and 4b). The epithelial cells had no cilia on their apical surfaces. Concentric whirling structures were formed by the spindle-shaped cells, and vascular structures were occasionally detected in the center of them (Fig. 4a). The stromal component containing spindle-shaped cells was highly cellular, with varying amounts of collagen fibers seen between the cells. Both the epithelial and stromal cells had bland nuclei, which did not display atypia, and mitoses were not detected in ≥10 high-power fields. A small number of multinucleated stromal cells were observed. The proportion of epithelial and stromal components remained unchanged throughout the lesion. Sparse focal infiltrates of lymphocytes and neutrophils were seen. No areas of necrosis or hemorrhaging were noted.

Immunohistochemistry was performed to determine the characteristics of the epithelial and stromal cells. We used the avidin-biotin-peroxidase complex procedure (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA, U.S.A.). The primary antibodies used included mouse monoclonal antibodies against pancytokeratin, cytokeratin 7, thyroid transcription factor-1,
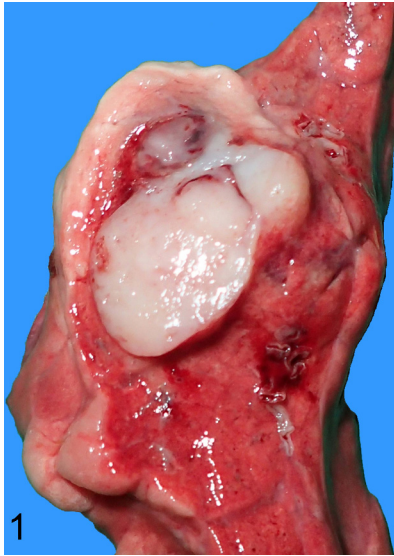
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Fig. 1. The cut surface of a pulmonary adenofibroma in a sika deer. A well-demarcated, tan-pink, rubbery nodule in the lung parenchyma.
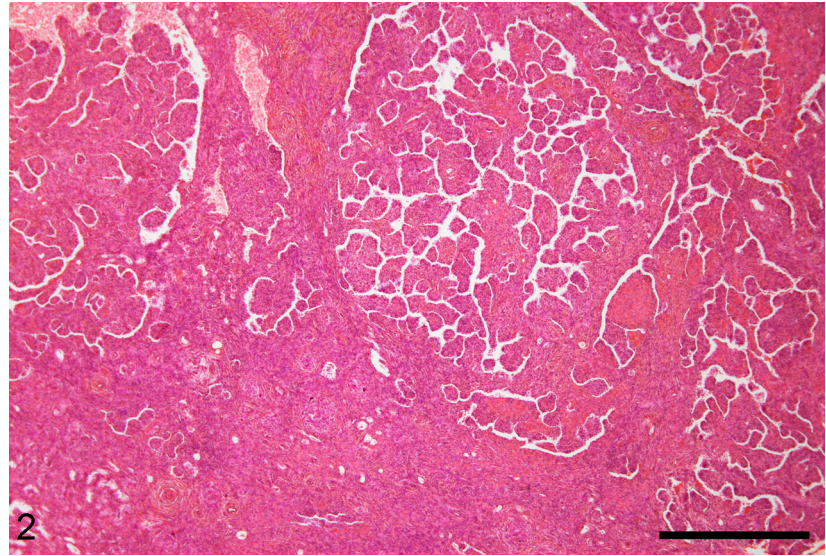
Fig. 2. Proliferating spindle-shaped cells exhibiting leaf-like and patternless growth patterns. Hematoxylin and eosin (HE). Bar=1 mm.
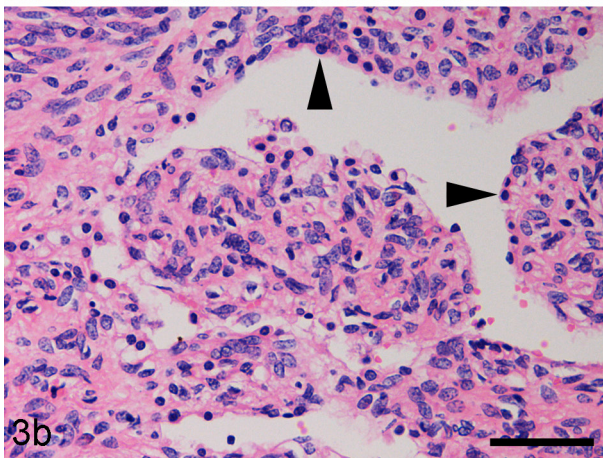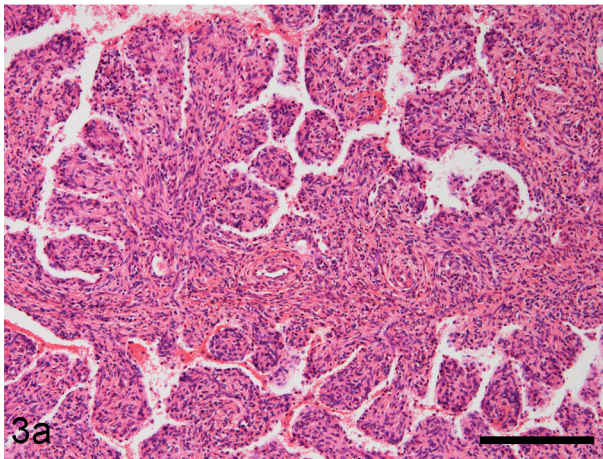
Fig. 3. a. Proliferating spindle-shaped cells that formed multiple, small, club-shaped projections into the lumen, resulting in staghorn-like, branching spaces. HE. Bar=200 μm. b. Slit-like spaces lined by a single layer of flattened to cuboidal epithelial cells (arrowheads). HE. Bar=50 μm.
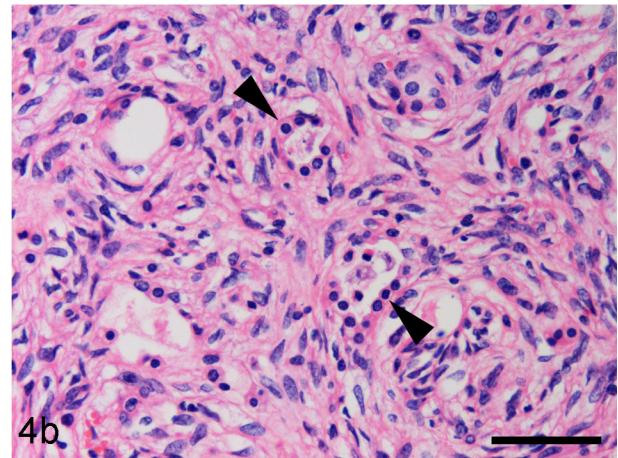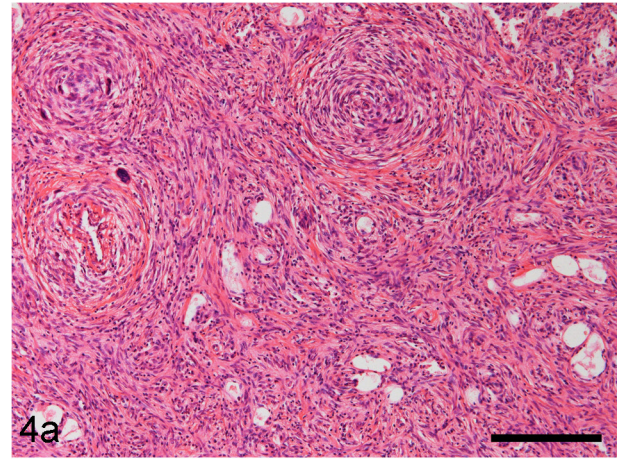
Fig. 4. a. Haphazardly proliferating spindle-shaped cells with isolated, small, gland-like spaces and concentric whirling structures. HE. Bar=200 μm. b. Small gland-like spaces lined by a single layer of flattened to cuboidal epithelial cells (arrowheads). HE. Bar=50 μm.

Table 1. Primary antibodies used for immunohistochemistry of a pulmonary adenofibroma in a sika deer

Antibody	Type (Clone)	Dilution	Antigen retrieval	Source
Pancytokeratin	Mouse, mAb (AE1/AE3)	Prediluted	MW	Nichirei, Tokyo, Japan
Cytokeratin 7	Mouse, mAb (RCK105)	1:100	AC	Abcam, Cambridge, UK
Napsin A	Rabbit, mAb (BC15)	1:100	AC	Biocare Medical, Pacheco, CA, U.S.A.
TTF-1	Mouse, mAb (8G7G3/1 & NX2.1/690)	1:100	AC	Novus Biologicals, Littleton, CO, U.S.A.
Vimentin	Mouse, mAb (V9)	1:100	AC	Dako, Glostrup, Denmark
Desmin	Mouse, mAb (D9)	1:80	MW	Progen, Heidelberg, Germany
α-SMA	Mouse, mAb (1A4)	1:100	MW	Dako, Glostrup, Denmark
Heavy caldesmon	Mouse, mAb (N5/22)	1:100	AC	Chemicon, Temecula, CA, U.S.A.
Factor 8	Rabbit, pAb	Prediluted	PR	Nichirei, Tokyo, Japan
S-100	Rabbit, pAb	1:300	–	Dako, Glostrup, Denmark
CD34	Mouse, mAb (QBEnd 10)	1:50	AC	Dako, Glostrup, Denmark
CD34	Goat pAb	1:50	AC	Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.
Ki-67	Mouse, mAb (MIB-1)	1:100	AC	Dako, Glostrup, Denmark

AC, autoclave at 121°C for 15 min in 10 mM citrate buffer, pH6.0; α-SMA, α-smooth muscle actin; mAb, monoclonal antibody; MW, microwave at 500 W for 15 min in 10 mM citrate buffer, pH6.0; pAb, polyclonal antibody; PR, treated with protease (Nichirei, Tokyo, Japan) at room temperature for 15 min; TTF-1, thyroid transcription factor-1.

Table 2. Immunohistochemical results obtained for a pulmonary adenofibroma of a sika deer and a postive control

Antibody	Epithelial lining cell	Stromal cell	Positive control in the lung of sika deer[a]
Pancytokeratin	++	–	Bronchial, bronchiolar, and type I and II aleveolar epithelial cells
Cytokeratin 7	+	–	Bronchial, bronchiolar, and type II aleveolar epithelial cells
Napsin A	+	–	Type II aleveolar epithelial cells
TTF-1	+	–	Bronchiolar and type II alveolar epithelial cells
Vimentin	–	++	Interstitial fibroblasts
Desmin	–	+	Muscluar layers of bronchus, bronchiolus and arteries
α-SMA	–	+	Muscluar layers of bronchus, bronchiolus and arteries
Heavy caldesmon	–	–	Muscluar layers of bronchus, bronchiolus and arteries
Factor 8	–	–	Vascular endothelial cells
S-100	–	–	Chondocytes in bronchial cartilage
CD34	NR	NR	NR
Ki-67[b]	0.28	0.45	Keratinocytes in skin

++, diffusely positive; +, partially positive; –, negative; NR, not reactive; α-SMA, α-smooth muscle actin; TTF-1, thyroid transcription factor-1. a) We used normal lung tissues from the same deer for a positive control, except for Ki-67. We used normal skin from another sika deer as a positive control for Ki-67. b) The results for Ki-67 indicate the number of nuclei that were positive for Ki-67 per 100 nuclei.

vimentin, desmin, α-smooth muscle actin, heavy caldesmon, and Ki-67; a rabbit monoclonal antibody against napsin A; and rabbit polyclonal antibodies against factor 8 and S-100. We also used mouse monoclonal and goat polyclonal antibodies against CD34. Detailed information about each antibody is provided in Table 1. We used 3, 3′-diaminobenzidine as a chromogen and counterstaining was performed with hematoxylin. The number of nuclei that were positive for Ki-67 was counted among over 500 epithelial cell nuclei and 2,000 stromal cell nuclei. The immunohistochemical results, including those for the positive control for each antibody are provided in Table 2. The epithelial lining cells were positively stained for pancytokeratin (Fig. 5), cytokeratin 7, napsin A, and thyroid transcription factor-1. Pancytokeratin was diffusely expressed in the flattened and cuboidal epithelial cells, and the antibodies against cytokeratin 7 and napsin A mainly labeled the cuboidal cells rather than the flattened cells. In the normal lung of the same animal, cytokeratin 7 was expressed in the bronchial, bronchiolar, and type II alveolar epithelia and napsin A was expressed in the type II alveolar epithelia. Weak immunoreactivity for thyroid transcription factor-1 was detected in the nuclei of the cuboidal epithelial cells in the lesion and in the nuclei of the bronchiolar and type II alveolar epithelial cells in the normal lung. The stromal cells were diffusely positive for vimentin (Fig. 6) and partially positive for desmin and α-smooth muscle actin, but negative for heavy caldesmon, factor 8, and S-100. The antibodies against CD34 did not work in our specimens. Among the epithelial and stromal cells, 0.29 and 0.45 per 100 nuclei were positive for Ki-67, respectively. Based on these findings, the lesion was revealed to be composed of epithelial and stromal cell components, both of which proliferated very slowly and exhibited low cellular and nuclear malignancy.

According to the veterinary literature, the differential diagnoses for biphasic lesions in the lungs include pulmonary blastoma, carcinosarcoma, and sarcoma with entrapment of normal epithelial structures. Pulmonary blastomas are rare lesions, which involve aggressive growth of mixed cell populations, and have been reported in cattle, a dog, and a laboratory rat [13]. In pulmonary blastomas, both epithelial and mesenchymal cells exhibit a primitive blast-like appearance, which mirrors their appearance
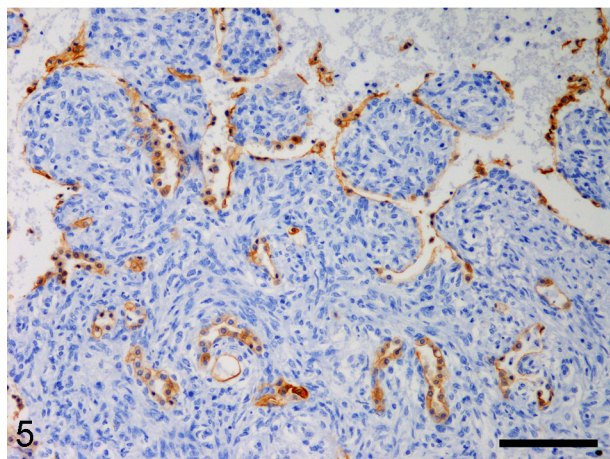
Fig. 5. The epithelial cells lining the slit-like and gland-like cavities are immunohistochemically positive for pancytokeratin. Bar=100 μm.
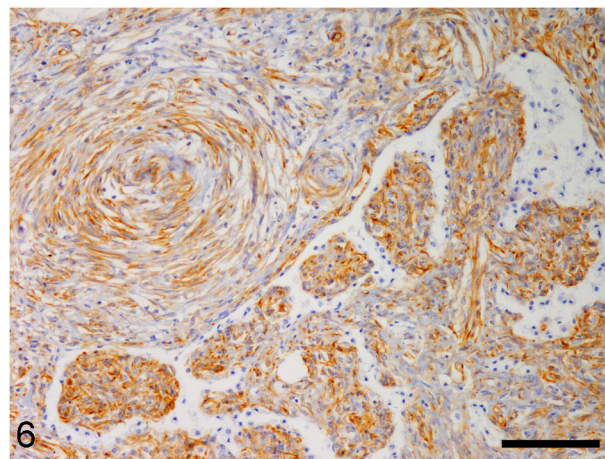

Fig. 6. The stromal spindle-shaped cells are immunohistochemically positive for vimentin. Bar=100 μm.

during embryonic pulmonary development. These findings differ from the bland cell appearance seen in the present lesion. Carcinosarcomas are composed of proliferating carcinomatous and sarcomatous components, and both elements display obvious features of malignancy. Thus, carcinosarcomas can also be distinguished from our case, which exhibited low cellular and nuclear malignancy. Sarcomas can entrap normal respiratory epithelia, which are usually arranged haphazardly and located at the periphery of such lesions. This is in stark contrast to the diffuse distribution of epithelial elements and the complex arrangement of epithelial and stromal structures seen in the current case. In this way, the histological and immunohistochemical findings of our case were not consistent with any lesions reported in the veterinary literature; however, they were similar to those of human pulmonary adenofibroma.

Pulmonary adenofibroma is an extremely rare lesion in humans. It was first described in 1944 [8], and few cases have been reported so far [1–6, 9–12]. Pulmonary adenofibromas usually occur as single well-demarcated nodules, ranging in size from 0.5 to 9.5 cm (mean maximum dimension: 2.9 cm), in the lung parenchyma [1–4, 6, 8–12]. One patient was reported to have 10 pulmonary adenofibromas, which ranged in size from 0.2 to 1.5 cm [5]. Histologically, pulmonary adenofibromas are biphasic lesions composed of epithelial and stromal components, and they exhibit a characteristic leaf-like/"phyllodes-like" growth pattern [1–6, 8–12]. The stromal components of pulmonary adenofibromas contain multiple, club-shaped papillary projections, which result in staghorn-like slit-shaped spaces lined by simple cuboidal to columnar epithelia [1–6, 8–12]. The stromal components of pulmonary adenofibromas are composed of bland spindle-shaped cells [1–6, 8–12], and their cell density varies from case to case and from region to region within the lesion (from highly cellular components composed of proliferating cells [4–6, 8, 11] to a densely sclerotic stroma with scattered spindle-shaped cells [1–4, 6, 9, 10, 12]). Isolated gland-like spaces with epithelial lining cells are present in the stroma [5, 6, 10–12]. Both the epithelial and stromal cells of pulmonary adenofibromas have bland nuclei, which do not exhibit obvious atypia or mitotic activity [1–6, 8–12]. The leaf-like fibroepithelial pattern was reported to resemble adenofibroma of the female genital tract or fibroadenoma of the breast [1, 3, 6, 8, 10, 11]. Immunohistochemically, the epithelial cells of pulmonary adenofibromas are positive for pancytokeratin, epithelial membrane antigen, thyroid transcription factor-1, cytokeratin 7, and napsin A [1–6, 9–12]. The stromal cells of pulmonary adenofibromas express vimentin and CD34 in most cases [1–6, 9–12], and variable expression of desmin, α-smooth muscle actin, CD99, and Bcl-2 has been noted [2, 4–6, 9, 11]. One study interpreted the immunohistochemical properties of pulmonary adenofibroma stromal cells as being indicative of focal differentiation towards smooth muscle [11]. The histological and immunohistochemical findings of our case were similar to those of pulmonary adenofibromas reported in humans; therefore, we diagnosed the larger nodule in the lung of the sika deer as a pulmonary adenofibroma. In the present case, the stromal spindle-shaped cells exhibited diffuse immunoreactivity for vimentin and focal immunoreactivity for desmin and α-smooth muscle actin, but were negative for heavy caldesmon, which confirmed that they had differentiated towards the myofibroblastic phenotype [7]. The concentric whirling arrangement of stromal cells found in our case has not been described in human cases. Although the significance of this finding with regard to the diagnosis of pulmonary adenofibroma is unclear, we considered it to be one of the variations seen in the arrangement of pulmonary adenofibroma stromal cells.

All of the reported cases of pulmonary adenofibroma involved adults, mostly individuals in their 6th decade of life (mean age: 57.2, range: 25–75 years), and similar frequencies of the condition were seen in both genders [1–6, 8–12]. Some cases were asymptomatic and were incidentally detected on chest radiographs or computed tomography scans [2, 3, 5, 8, 10], and the other cases involved chest pain or hemoptysis [1, 6, 11, 12]. Radiographically, pulmonary adenofibromas appear as solitary nodular lesions [1, 3–6, 9–12]. In all of the reported cases except for a case which was found postmortem [8], the lesions were surgically resected, and no evidence of postoperative recurrence or metastasis was reported [1–6, 9–12]. In our case, because the lesion was

incidentally detected at slaughter, no clinical or clinicopathological findings were available.

The histogenesis of pulmonary adenofibroma has been a subject of controversy, with some researchers maintaining that pulmonary adenofibroma has a hamartomatous nature [1, 6, 8, 10] and others suggesting that it is a true neoplastic lesion [2, 9, 11, 12]. Recently, Fusco *et al*. detected a highly recurrent NGFI-A-binding protein 2 (NAB2)-signal transducer and activator of transcription 6 (STAT6) fusion variant in the stromal components of 5 of the 7 pulmonary adenofibroma cases they examined [4]. The NAB2-STAT6 fusion gene is specific to solitary fibrous tumors in humans, and therefore, they concluded that pulmonary adenofibromas are neoplastic lesions [4]. In the current case, morphologically, the epithelial component was composed of a single layer of flattened to cuboidal cells. The flattened cells were immunohistochemically positive for pancytokeratin, and the cuboidal cells were positive for cytokeratin 7 and napsin A. These properties of the epithelial component were consistent with those of the normal bronchiolar to alveolar epithelia. In addition, the proportion of epithelial and stromal components remained unchanged throughout the lesion, and the proliferative activity of both components was very low. From these observations, we considered that the pulmonary lesion in the present case was likely to be a hamartoma composed of epithelial and stromal components.

In this paper, we reported a case of pulmonary adenofibroma in a sika deer, which, to the best of our knowledge, is the first reported case of pulmonary adenofibroma in an animal. We should include adenofibroma in the differential diagnoses for biphasic pulmonary lesions with epithelial and stromal components.

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