Case Report

Bronchioloalveolar Carcinoma in an Aged Beagle Dog

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Abstract: A pulmonary mass was observed in a 16-year-old beagle dog. In the histopathology, a well-circumscribed mass composed of cuboidal to low or tall columnar neoplastic cells proliferating in a papillary, acinar or solid pattern and abundant cholesterol deposition was observed. The tumor cells had eosinophilic to abundant clear or foamy cytoplasm. The cells were arranged around blood vessels with the nuclei at the periphery of the cells in a pseudo-rosette formation, especially in the tall columnar cells. In the immunohistochemistry, the neoplastic cells were positive for cytokeratin and surfactant apoprotein A, but not for vimentin and chromogranin A. In the electron microscopy, numerous vacuoles ranging from 0.5 to 2 μ m in diameter were observed in the basal part of the neoplastic cells and some of these vacuoles contained myelin-like materials. These findings indicate that these neoplastic cells were derived from alveolar type II cells. Therefore, the pulmonary mass was diagnosed as bronchioloalveolar carcinoma. (*J Toxicol Pathol* 2008; **21**: 109–113)

Key words: bronchioloalveolar carcinoma, lung, dog

The frequency of pulmonary tumors in domestic animals is low and depends on the species. Primary lung tumors in dogs are generally considered to be of low frequency, although tumors that have metastasized to the lung are relatively common^{1,2}. It is important to distinguish between primary lung tumors and metastatic tumors, although it is difficult to differentiate metastatic tumors from primary tumors by their microscopic appearance².

Most primary lung tumors in dogs are epithelial in origin and adenocarcinoma is the most frequent³. Adenocarcinoma of the lung is classified based on its origin. The origin of papillary adenocarcinoma is in the large airway and it tends to grow near the hilus in large aggressive masses. The origin of bronchioloalveolar carcinoma is in the peripheral airways, such as the bronchiolar or alveolar regions. It is difficult to distinguish between these two carcinomas based on routine histopathological examinations, especially in the case of less-advanced tumors.

In this report, we describe a case of bronchioloalveolar carcinoma in an aged Beagle dog which had been maintained for an extended period without undergoing any toxicity studies, in order to collect clinical and pathological background data in a Beagle colony.

A colony of Beagle dogs, including the case in this report, was maintained for a long period under proper breeding conditions at our facility. The care and treatment guidelines were approved by the Ethics Review Committee for Animal Experimentation of Daiichi Sankyo Co., Ltd. A 16-year-old male Beagle dog underwent euthanasia after exhibiting a decrease in food consumption, emaciation and a decrease in activity.

In the gross pathology, a distinct whitish nodule, approximately 20 mm in diameter, was observed in the right medial lobe of the lung, but there were no similar lesions in the other organs. Hepatization of the left anterior and posterior lobes was also observed. All the samples were fixed in neutral-buffered 10% formalin solution and embedded in paraffin by routine procedures. Each paraffin-embedded section of 2 or 3 μ m thickness was stained with hematoxylin-eosin (HE), PAS or oil red O (using frozen sections). Immunohistochemistry was performed using the Dako EnVision system (Dako Japan, Kyoto, Japan). All antibodies were purchased from Dako Japan. Each lung section was incubated with rabbit antisera against chromogranin A, with mouse monoclonal antibodies against vimentin (clone V9), cytokeratin (clone AE1/AE3) and surfactant apoprotein A (clone PE10)⁴. The reaction products were visualized with 3, 3'-diaminobenzidine tetrahydrochloride. For the electron microscopic examination, the formalin-fixed lung tissue was fixed in 2% glutaraldehyde and 2% paraformaldehyde, and then 2% osmium tetroxide. The tissue samples were embedded in resin (Poly/Bed 812: Polysciences Inc., Japan) and ultrathin

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Fig. 1. The well-circumscribed nodule was divided into several lobes by distinct fibrous tissues. Prominent deposition of cholesterol crystals was observed. HE stain, $\times 10$.

sections were prepared with an ultramicrotome (Reichert Ultracut S, Leica AG, Austria), stained with uranum acetate and lead citrate, and observed with a transmission electron microscope (JEM-2010, JEOL Ltd., Japan).

In the histopathological examination, the nodule was well-circumscribed and divided into several lobes by distinct fibrous stroma (Fig. 1). The tumor cells were composed of cuboidal to low or tall columnar cells in a papillary, acinar or solid pattern and were arranged in a line (Fig. 2). The tumor cells destroyed the alveolar septa and invaded the surrounding tissue. The tumor cells had eosinophilic to abundant clear or foamy cytoplasm. The neoplastic cells were round and basophilic in the cuboidal cells (Fig. 2A), and there were clear nuclei in the low to tall columnar cells with less mitotic figures. The cells were quite often arranged around blood vessels with the nuclei at the periphery of the cells in a pseudo-rosette formation, especially in the tall columnar cells (Fig. 2C). In the neoplastic nodule, granuloma associated with abundant cholesterol deposition (Fig. 1), necrotic foci, aggregation of brown pigment loaded macrophage and mineral deposition were observed. There were no neoplastic lesions in any other examined organs, except for a leiomyoma in the stomach and Leydig cell tumor in the testis. The cytoplasm of the neoplastic cells was negative for PAS and oil red O stain, although goblet cells in the bronchial epithelium were positive for PAS stain and the macrophages aggregated around the cholesterol crystals were positive for oil red O stain.

In the immunohistochemical examinations, the tumor cells were positive for cytokeratin (Fig. 3A) and negative for vimentin (Fig. 3B), although macrophages in the alveolar space and surrounding the cholesterol deposition were strongly positive for vimentin. Clear or foamy cytoplasm of tall columnar cells were negative for chromogranin A (Fig. 3C), however, surfactant apoprotein A was weakly positive

Fig. 2. The tumor cells were composed of cuboidal to low or tall columnar cells in a papillary, acinar or solid pattern and were arranged in a line. A) Small cuboidal tumor cells with basophilic nuclei and cytoplasm. B) Tall columnar tumor cells with basophilic nuclei and foamy cytoplasm. The nuclei of the cells were located at the periphery of the cells. C) Tall columnar tumor cells with clear nuclei and foamy cytoplasm. The nuclei of the cells were located at the periphery of the cells. Necrotic cells were observed in the alveolar space. HE stain, $\times 215$.

Fig. 3. Immunohistochemistry of the tumor. A) cytokeratin, B) vimentin, C) chromogranin A, and D) surfactant apoprotein A. The tumor cells were positive for cytokeratin and surfactant apoprotein A. $\times 215$.

in cytoplasm and the cytoplasmic membrane of the neoplastic cells (Fig. 3D) and in the alveolar space and brown pigment loaded macrophages.

In the electron microscopic examination, numerous vacuoles ranging from 0.5 to 2 μm in diameter were observed in the basal part of the tall columnar neoplastic cells. Some of these vacuoles contained myelin-like materials (Fig. 4). The mitochondria of the neoplastic cells did not show either vesicular or trabecular type cristae, typical features of endocrine cells.

The peripheral location of the nuclei of the neoplastic cells and the prominent accumulation of cholesterol deposition were characteristic in this case. The nuclei location suggested the possibility that they might be endocrine tumors such as neuroendocrine/carcinoid tumors or metastasis of Leydig cell tumors. Neuroendocrine/carcinoid tumors are derived from the dispersed neuroendocrine system and are characterized by granular eosinophilic or clear cytoplasm and ultrastructurally small dense-core neurosecretory granules in their cytoplasm. In

the present case, the tumor cells were negative for chromogranin A, a functional marker of neuroendocrine tumors, and they did not have neuroendocrine granules ultrastructurally.

In the present case, Leydig cell tumors were observed in the testis and the proliferation patterns of the lung tumors were similar to the solid-diffuse pattern of Leydig cell tumors^{1,5}. In a solid-diffuse pattern, tumor cells have eosinophilic, coarsely or finely vacuolated cytoplasm that contain lipid, proliferate in sheets or cords separated by thin connective tissue and often show rosette formations around the vessels⁶. However, the lung tumor cells in the present case were negative for oil red O stain and positive for cytokeratin and they did not show the vesicular or trabecular type cristae of mitochondria, which are characteristic to endocrine cells. These findings suggest that the tumor cells were derived from epithelial cells in the lung and not from endocrine cells.

The WHO guideline classifies adenocarcinomas in the lung as those whose origin is not evident from their

Fig. 4. Electron microscopy of the tumor. Numerous vacuoles ranging from 0.5 to 2 μm in diameter, which contained myelin-like materials were observed in the basal part of the tall columnar tumor cells. $\times 5500$.

histological features and those with features indicating a probable origin in the bronchiolar and/or alveolar region¹. Papillary adenocarcinoma is derived from the large airway, tends to grow near the hilus, and is divided into lobules by thick connective tissue septa. The cells are medium to tall columnar and have large, ovoid basal nuclei and are often stratified. Papillary adenocarcinomas are usually highly destructive and invasive. Bronchioloalveolar carcinoma is often observed in the peripheral region of the lung and is derived from alveolar type II cells and/or Clara cells. The structural features of these tumors include a regular alveolar pattern and the preservation of their alveolar architecture. The cells are cuboidal to low columnar and are less stratified. In the present case, the morphological patterns were highly destructive and similar to papillary adenocarcinoma, but the immunohistochemical and ultrastructural findings showed that the tumor cells were positive for surfactant apoprotein antibody and had myelin-like bodies in the cytoplasm, suggesting that they were of alveolar type II cell origin. Therefore, we diagnosed it as bronchioloalveolar carcinoma.

In the present case, few mitotic figures were observed. However, destruction of the alveolar septa, invasion into the surrounding tissue, and vast necrotic foci were evidence of malignant transformation. In particular, atypia of the nucleus locations were highly observed in the present case. The reason why the nuclei of the tumor cells derived from epithelial cells were located in the periphery of the cells was not elucidated, but we consider it to be evidence of malignancy. As normal alveolar type II cells are characteristically multipolar and are often facing the

adjacent alveolar space⁷, these cells may tend to cause atypia of the nucleus locations more than other epithelial tumors.

Abundant cholesterol deposition was also a characteristic of the tumor in this case. As alveolar type II epithelial cells contain abundant surfactant, this finding suggested that the tumor cells were derived from alveolar type II cells. It has been reported that necrotic regions frequently contain the foci of cholesterol cleft formation in cases of bronchioloalveolar carcinoma⁸. The formation of thick connective tissue surrounding the nodules was considered to be related to cholesterol granuloma.

It has been reported that the incidence of primary lung tumors is low in young dogs, but that it is higher in aged dogs and is about 18 to 25% in dogs over 16 years of age⁹. Bronchioloalveolar carcinoma is more common in dogs^{8,10}. However, in the present case we observed an atypical location of the nuclei of the tumor cells, which has not been reported in other lung tumors. Consequently, we consider the present case to be a rare case.

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References

1. Dungworth DL, Hauser B, Hahn FF, Wilson DW, Haenichen T, and Harkema JR. Histological classification of tumors of the respiratory system of domestic animals. In: *The WHO International Histological Classification of Tumors of Domestic Animals*. 2nd series. Vol. VI, Armed Forces Institute of Pathology, Washington D.C. 1999.
2. TenHave-Opbroek AA, Shi XB, and Gumerlock PH. 3-Methylcholanthrene triggers the differentiation of alveolar tumor cells from canine bronchial basal cells and an altered p53 gene promotes their clonal expansion. *Carcinogenesis*. **21**: 1477–1484. 2000.
3. Peters MA, Teerds KJ, van der Gaag I, de Rooij DG, and van Sluijs FJ. Use of antibodies against LH receptor, 3 β -hydroxysteroid dehydrogenase and vimentin to characterize different types of testicular tumour in dogs. *Reproduction*. **121**: 287–296. 2001.
4. Grossman DA, Hiti AL, McNiel EA, Ye Y, Alpaugh ML, and Barsky SH. Comparative oncological studies of feline bronchioloalveolar lung carcinoma, Its derived cell line and xenograft. *Cancer Res*. **62**: 3826–3833. 2002.
5. Taniyama H, Hirayama K, Nakada K, Numagami K, Yaosaka N, Kagawa Y, Izumisawa Y, Nakade T, Tanaka Y, Watanabe G, and Taya K. Immunohistochemical detection of inhibin- α , - β B, and - β A chains and 3 β -hydroxysteroid dehydrogenase in canine testicular tumors and normal testes. *Vet Pathol*. **38**: 661–666. 2001.
6. Kennedy PC, Cullen JM, Edwards JF, Goldschmidt MH, Larsen S, Munson L, and Nielsen S. Histological classification of tumors of the genital system of domestic animals. In: *The WHO International Histological Classification of Tumors of Domestic Animals*. 2nd series. Vol. VI, Armed Forces Institute of Pathology, Washington D.C. 1998.

7. Yokoyama T. Current Encyclopedia of Pathology, Vol. 14A, Respiratory system I, Nakayama-Shoten Co., Ltd., Tokyo. 1993.
8. Wilson DW and Dungworth DL. Tumors of the respiratory tract. In: Tumors in Domestic Animals, 4th ed. Meuten DJ (ed). Iowa State Press, Iowa. 365–400. 2002.
9. Hahn FF, Muggenburg BA, and Griffith WC. Primary lung neoplasia in a beagle colony. *Vet Pathol.* **33**: 633–638. 1996.
10. Griffey SM, Kraegel SA, and Madewell BR. Rapid detection of K-ras gene mutations in canine lung cancer using single-strand conformational polymorphism analysis. *Carcinogenesis.* **19**: 959–963. 1998.