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Mucinous Cystadenoma in the Lung of a Captive-born Moustached Tamarin (*Saguinus mystax*)

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Summary

A 2-year-old captive-born male moustached tamarin was subjected to necropsy examination after a fatal head trauma. A solitary, circumscribed, subpleural mass (0.6 cm diameter) was found in the right caudal lung lobe. The mass was diagnosed as a mucinous cystadenoma. Histochemical and immunohistochemical tests were performed to further characterize the tumour. Surfactant proteins A, B, C and D were not found in the neoplastic cells, suggesting that the tumour arose from a non-surfactant producing alveolar lining cell. Pulmonary mucinous cystadenomas are uncommon benign tumours in man and have not been reported previously in animals.

Keywords

pulmonary cystadenoma; surfactant proteins; immunohistochemistry; moustached tamarin

Cystadenomas are epithelial tumours that are formed by the cystic dilation of secretory glandular epithelial units and may arise in multiple organs. Cystadenomas are subclassified by their form (e.g. serous, papillary or mucinous) or by their location (e.g. bile duct, endometrium, appendix or pancreas). Mucinous cystadenoma is a tumour that forms a uni- or multilocular mass lined by well-differentiated cuboidal to columnar mucus-producing epithelium (Head and Else, 2002; Gao and Urbanski, 2005). The World Health Organization defines mucinous cystadenoma as a localized cystic mass filled with mucin and surrounded by a fibrous wall lined by well-differentiated columnar mucinous epithelium (Travis *et al.*, 2004). Mucinous cystadenomas have been described in the parotid gland (Head and Else, 2002) and gall bladder in cats (Cullen and Popp, 2002) and in the ovary of a cow (García-Iglesias *et al.*, 1991) and a cynomolgus macaque (Sato *et al.*, 2008). There are no reports of the mucinous form of cystadenoma in the lungs of mammals other than man. This report describes a mucinous cystadenoma in the lung of a non-human primate.

A 2-year-old, male, captive-born, moustached tamarin (*Saguinus mystax*) was part of a study into viral hepatitis that was approved by the Institutional Animal Care and Use Committee and was completed at the time of the animal's death. The tamarin was pair-

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housed with a female, in accordance with advice and regulations (Institute for Laboratory Animal Research, 1996). The tamarin was found dead on morning rounds and a complete necropsy examination was performed. The carcass was in good bodily condition. A single large area of haemorrhage was noted at the base of the skull. This lesion was indicative of compression head trauma, which was ruled as the cause of death and attributed to cage-mate fighting. An incidental finding was a round, well-demarcated, subpleural, circumscribed mass (0.6 cm diameter) in the distal portion of the right caudal lung lobe. Tissue samples were collected from all major organs and fixed in 10% neutral buffered formalin. Following routine processing and embedding in paraffin wax, sections were stained with haematoxylin and eosin (HE). Sections of the lung mass were also stained with periodic acid–Schiff (PAS) with and without diastase digestion, alcian blue (pH 2.5), toluidine blue, Mayer's mucicarmine, Gram stain, Masson's trichrome (MT) and elastic Van Gieson stains.

Immunohistochemistry (IHC) was performed as described by Michaud *et al.* (2012) using antibodies against surfactant protein (SP)-A (Millipore, Temecula, California, USA; rabbit polyclonal antibody reactive with human, mouse, rat and primate SP-A; dilution 1 in 500), SP-B (Abcam, Cambridge, Massachusetts, USA; rabbit polyclonal antibody reactive with mouse, sheep, cow and human SP-B; dilution 1 in 500), SP-C (Abcam; rabbit polyclonal antibody reactive with human and mouse SP-C; dilution 1 in 100) and SP-D (Abcam; biotinylated mouse monoclonal antibody specific for human and rat SP-D; clone IIE11; dilution 1 in 40). Positive and negative controls were used in each experiment.

Microscopically, the mass was well-circumscribed and non-encapsulated, located within the subpleural space, causing a nodular expansion and compressing the adjacent lung parenchyma with no evident invasive growth (Fig. 1). The mass was composed of randomly-arranged cystic spaces, which were up to 40 times the size of non-neoplastic lung alveoli, lined mostly by a single layer of columnar to lower cuboidal mucus-secreting epithelial cells, with occasional small papillary projections into the lumen. The mucus-producing cells had a single round to polygonal basally-oriented nucleus, containing up to two densely basophilic nucleoli and had a stippled chromatin pattern with minimal atypia and no mitotic figures. In some cells, the nuclei were hyperchromatic and homogeneous. Few areas with cells undergoing apoptosis were observed in the epithelium lining the cystic spaces. The branching trabecular stroma that supported and divided the mass into cystic spaces was variably attenuated, often composed of spindle-shaped cells with an elongated thin nucleus, loose connective tissue, some medium-sized blood vessels and infiltrates of low numbers of lymphocytes and plasma cells in some areas; however, some trabeculae consisted of only a monolayer of cuboidal to flattened cells resembling interalveolar septa and lacking the lining of mucus producing cells (Fig. 2). The cystic spaces within the mass were filled with an eosinophilic, granular material that was loosely arranged in whorls, cell remnants and variable numbers of foamy macrophages. Masson's trichrome staining demonstrated mucin within the cystic spaces and minimal stromal collagen. The granular material was PAS-positive and diastase-resistant (Fig. 3), displaying positive reaction with the alcian blue and Mayer's mucicarmine stains. A discrete area at the periphery of the mass showed extension of this material towards the adjacent normal lung parenchyma (Fig. 1). None of the SPs was expressed immunohistochemically by the neoplastic epithelium. A discrete and small area in the middle of the tumour reacted strongly with antibody to SP-B, suggesting trapped surfactant (Fig. 4). The normal adjacent lung parenchyma displayed SP-A, SP-B, SP-C and SP-D in type II pneumocytes and alveolar macrophages, in addition to a free form of surfactant containing these four SPs that lined the alveolar spaces.

Mucinous cystic tumours of the lung in man are uncommon and there are fewer than 100 reports in the literature (Davison *et al.*, 1992; Guimaraes *et al.*, 2004; Haro-Estarriol *et al.*, 2004; Gao and Urbanski, 2005; Igai *et al.*, 2008; Haruki *et al.*, 2010). Three variants of

mucinous cystadenomas are described, including benign mucinous cystadenoma, mucinous cystic tumour with borderline malignancy and mucinous cystadenocarcinoma (Graeme-Cook and Mark, 1991; Davison *et al.*, 1992; Gao and Urbanski, 2005; Iwasaki *et al.*, 2007). Benign mucinous cystadenomas are well-demarcated, solitary, unilocular or multilocular cystic masses, often but not always surrounded by a fibrous wall. The cystic spaces, which are lined by a well-differentiated cuboidal to columnar mucus-producing epithelium are filled with a few cell remnants and scant to abundant mucin (Roux *et al.*, 1995; Matsuo *et al.*, 2005; Igai *et al.*, 2008; Haruki *et al.*, 2010), the latter component representing up to 90% of the tumour (Gao and Urbanski, 2005; Haruki *et al.*, 2010). Mucinous cystic tumours with borderline malignancy have been described as predominantly having a tall mucinous stratified epithelium with cytological atypia, hyperchromatic and pleomorphic nuclei, occasional mitotic figures, foci of cells heaped into tufts or papillae, mucin dissecting the surrounding lung parenchyma, sometimes lymphoplasmacytic infiltrates, and fibrosis (Graeme-Cook and Mark, 1991; Mann *et al.*, 2001; Haro-Estarriol *et al.*, 2004), but with no convincing evidence of malignant invasion (Davison *et al.*, 1992). Mucinous cystadenocarcinomas are more cellular and less cystic than the benign forms (Mann *et al.*, 2001). They exhibit necrosis and foci of malignant goblet cells (Roux *et al.*, 1995; Igai *et al.*, 2008), which are in small numbers in the periphery or in intramural septa (Gaeta *et al.*, 1999) and may have solid invasive areas present (Gao and Urbanski, 2005; Iwasaki *et al.*, 2007).

The World Health Organization classification system recognizes mucinous cystadenoma and mucinous cystadenocarcinoma, but not the intermediate mucinous cystic tumour of borderline malignancy (Travis *et al.*, 2004), despite its recognition as a separate entity in several reports (Graeme-Cook and Mark, 1991; Davison *et al.*, 1992; Mann *et al.*, 2001; Haro-Estarriol *et al.*, 2004; Gao and Urbanski, 2005; Matsuo *et al.*, 2005). In the present case, the lesion was diagnosed as a benign form of mucinous cystadenoma due to the uniformity, minimal cytological atypia and the absence of mitotic figures in the mucin-producing epithelium. The rapid growth of the mass in this case may have been attributed more to the production of mucin (Igai *et al.*, 2008) than to proliferation of the neoplastic epithelial cells. Additionally, the absence of a fibrous wall surrounding the present mass suggests recent development. This is consistent with the findings of Davison *et al.* (1992) who speculated that the presence of a fibrous wall was an indicator of longstanding nature. This point is disputed by others (Kragel *et al.*, 1990; Davison *et al.*, 1992; Dixon *et al.*, 1993; Roux *et al.*, 1995; Guimaraes *et al.*, 2004; Gao and Urbanski, 2005; Matsuo *et al.*, 2005; Igai *et al.*, 2008; Haruki *et al.*, 2010) who report the presence of a fibrous wall in some benign cystadenomas and absence of a fibrous wall in some malignant forms.

Characteristics that tend to favour a diagnosis of cystadenocarcinoma, such as focal thickening of the cystic wall and septae, cellular pleomorphism and the presence of mitotic activity (Gaeta *et al.*, 1999), were not observed in the present case. The present tumour was located in the right caudal lung lobe and right lobes are the most commonly reported location for the equivalent human tumours (Kragel *et al.*, 1990; Davison *et al.*, 1992; Roux *et al.*, 1995; Guimaraes *et al.*, 2004; Gao and Urbanski, 2005; Igai *et al.*, 2008; Haruki *et al.*, 2010). Additionally, the position of the present tumour (in the peripheral lung parenchyma and subpleural area) is characteristic of mucinous cystadenomas (Roux *et al.*, 1995; Guimaraes *et al.*, 2004; Matsuo *et al.*, 2005; Igai *et al.*, 2008; Haruki *et al.*, 2010). The granular material contained within the cysts and the cytoplasm of the epithelial lining was PAS-positive and diastase-resistant (Graeme-Cook and Mark, 1991; Dixon *et al.*, 1993; Roux *et al.*, 1995; Gao and Urbanski, 2005; Iwasaki *et al.*, 2007) and displayed prominent staining with alcian blue (Graeme-Cook and Mark, 1991; Roux *et al.*, 1995; Gao and Urbanski, 2005; Iwasaki *et al.*, 2007) and Mayer's mucicarmine (Graeme-Cook and Mark, 1991; Dixon *et al.*, 1993; Gao and Urbanski, 2005), confirming the presence of a mixture of

neutral (unstained portions) and carboxylated and sulphated mucins. The absence of immunoreaction for the four surfactant proteins in the cells lining the cystic spaces suggests that the tumour was derived from non-alveolar epithelium (Haruki *et al.*, 2010).

Mucinous cystadenomas have been reported previously to be SP-A negative (Kragel *et al.*, 1990; Haruki *et al.*, 2010). Mucinous cystadenomas of the lung must be differentiated from bronchial mucous gland adenomas, mucoepidermoid carcinomas, mucous bronchioalveolar carcinomas and metastatic adenocarcinomas (Kragel *et al.*, 1990; Dixon *et al.*, 1993). Bronchial mucous gland adenomas are polypoid endobronchial tumours of the tracheobronchial seromucinous glands (Mann *et al.*, 2001), composed of mucus-filled cystic glands with non-dilated microacini, tubules and papilla lined by a columnar to flattened secretory epithelium, supported by a thin spindle-cell stroma (Dixon *et al.*, 1993; Kragel *et al.*, 1990). In contrast, mucinous cystadenomas are located in the lung parenchyma rather than the bronchus and are macrocystic with abundant mucin secretion (Kragel *et al.*, 1990; Dixon *et al.*, 1993; Gao and Urbanski, 2005). Mucoepidermoid carcinomas, which arise from the submucosal bronchial glands, are characterized by mixtures of mucinous glandular cells, squamoid cells and intermediate cells forming circumscribed microcystic tumours; the epidermoid elements being the main feature (Kragel *et al.*, 1990; Roux *et al.*, 1995; Mann *et al.*, 2001; Gao and Urbanski, 2005). The mucinous forms of bronchioalveolar cell carcinoma lack a cyst wall and show nuclear pleomorphism and lepidic spread of malignant cells growing in a pneumonic pattern thorough the air spaces (Graeme-Cook and Mark, 1991; Davison *et al.*, 1992; Gaeta *et al.*, 1999; Guimaraes *et al.*, 2004; Gao and Urbanski, 2005; Igai *et al.*, 2008). This type of tumour can produce mucin and develop cysts, but exhibits alveolar spreading and metastases and has elastic tissue and smooth muscle in its wall (Roux *et al.*, 1995), all features absent in mucinous cystadenomas. Solitary metastatic nodules of adenocarcinomas, especially those of pancreatic, gastric, colonic, testicular or ovarian origin, could resemble mucinous cystadenoma (Kragel *et al.*, 1990; Davison *et al.*, 1992; Dixon *et al.*, 1993; Roux *et al.*, 1995; Gao and Urbanski, 2005), but in the present case, no primary neoplasia was present.

Non-neoplastic pulmonary cystic lesions that must be considered as differentials include developmental bronchogenic cysts, congenital adenomatoid malformations and post infectious bronchogenic cysts, all of which show foci of mucinous epithelium, but are more commonly lined by ciliated columnar epithelium (Kragel *et al.*, 1990). Developmental bronchogenic cysts are extrapulmonary, filled with either fluid or mucus, usually unilocular, solitary, and attached to the trachea or major bronchi. They generally have pseudostratified ciliated cells in the lining and smooth muscle, seromucinous glands or cartilage in the wall (Kragel *et al.*, 1990; Davison *et al.*, 1992; Roux *et al.*, 1995; Guimaraes *et al.*, 2004; Gao and Urbanski, 2005); these characteristics differentiate them from mucinous cystadenomas. Congenital cystic adenomatoid malformations are multiple, usually large cysts that extend towards the lung parenchyma. The cyst cavities are lined by cuboidal to pseudostratified epithelium and the wall usually contains smooth muscle and connective tissue, but no cartilage (Davison *et al.*, 1992; Roux *et al.*, 1995; Gao and Urbanski, 2005). Post infectious bronchogenic cysts communicate with or are close to bronchi (Kragel *et al.*, 1990; Davison *et al.*, 1992) and there was no evidence within the present case of cartilage or bronchial communication, which also suggests that the mass did not begin as an area of emphysema. No solid tumour areas were identified, or areas of necrosis and dystrophic calcification, within the lung mass in this tamarin.

Pulmonary mucinous cystadenomas are rare benign tumours with unique characteristics that distinguish them from developmental pulmonary mucinous cystic lesions and should be considered among the differentials for neoplasia of the lungs. To the authors' knowledge, this is the first report of this type of tumour in a non-human primate.

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References

- Cullen, JM.; Popp, JA. Tumors of the liver and gall bladder. In: Meuten, DJ., editor. Tumors in Domestic Animals. 4. Iowa State Press; Ames: 2002. p. 494
- Davison AM, Lowe JW, Da Costa P. Adenocarcinoma arising in a mucinous cystadenoma of the lung. Thorax. 1992; 47:129–130. [PubMed: 1549820]
- Dixon AY, Moran JF, Wesselius LJ, McGregor DH. Pulmonary mucinous cystic tumor. Case report with review of the literature. American Journal of Surgical Pathology. 1993; 17:722–728. [PubMed: 8317612]
- Gaeta M, Blandino A, Scribano E, Ascenti G, Minutoli F, et al. Mucinous cystadenocarcinoma of the lung: CT-pathologic correlation in three cases. Journal of Computer Assisted Tomography. 1999; 23:641–643. [PubMed: 10433300]
- Gao ZH, Urbanski SJ. The spectrum of pulmonary mucinous cystic neoplasia: a clinicopathologic and immunohistochemical study of ten cases and review of literature. American Journal of Clinical Pathology. 2005; 124:62–70. [PubMed: 15923171]
- García-Iglesias MJ, Martínez-Rodríguez JM, Bravo- Moral AM, Escudero-Diez A. Common epithelial tumours of the ovary in cows. Research in Veterinary Science. 1991; 50:358–359. [PubMed: 1882145]
- Graeme-Cook F, Mark EJ. Pulmonary mucinous cystic tumors of borderline malignancy. Human Pathology. 1991; 22:185–190. [PubMed: 2001880]
- Guimaraes AR, Wain JC, Mark EJ, Wittram C. Mucinous cystadenoma of the lung. American Journal of Roentgenology. 2004; 183:282. [PubMed: 15269012]
- Haro-Estarriol M, Baldó-Padró X, Casas-Tarrús M. Tumor mucinoso pulmonar de baja malignidad. Archivos de Bronconeumología. 2004; 40:426–427. [PubMed: 15458622]
- Haruki T, Nakamura H, Taniguchi Y, Miwa K, Adachi Y, et al. Pulmonary mucinous cystadenomas: a rare benign tumor. General Thoracic and Cardiovascular Surgery. 2010; 58:287–290. [PubMed: 20549459]
- Head, KW.; Else, RW. Other tumors of the alimentary tract. In: Meuten, DJ., editor. Tumors in Domestic Animals. 4. Iowa State Press; Ames: 2002. p. 412
- Igai H, Okumura N, Ohata K, Matsuoka T, Kameyama K, et al. Pediculate mucinous cystadenoma difficult to differentiate from pleural tumor. Annals of Thoracic Surgery. 2008; 85:1807–1809. [PubMed: 18442598]
- Institute for Laboratory Animal Research, National Research Council. Guide for the Care and Use of Laboratory Animals. National Academy Press; Washington, DC: 1996.
- Iwasaki T, Kawahara K, Nagano T, Nakagawa K. Pulmonary mucinous cystadenocarcinoma: an extremely rare tumor presenting as cystic lesion of the lung. General Thoracic Cardiovascular Surgery. 2007; 55:143–6. [PubMed: 17447515]
- Kragel PJ, Devaney KO, Meth BM, Linnoila I, Frierson HF Jr, et al. Mucinous cystadenoma of the lung. A report of two cases with immunohistochemical and ultrastructural analysis. Archives of Pathology and Laboratory Medicine. 1990; 114:1053–1056. [PubMed: 1699507]
- Mann GN, Wilczynski SP, Sager K, Grannis FW Jr. Recurrence of pulmonary mucinous cystic tumor of borderline malignancy. Annals of Thoracic Surgery. 2001; 71:696–697. [PubMed: 11235730]
- Matsuo T, Yusuke Kimura N, Takamori S, Shirouzu K. A case of recurrent pulmonary mucinous cystadenoma. European Journal of Cardiothoracic Surgery. 2005; 28:176–177. [PubMed: 15939602]
- Michaud CR, Ragland DR, Shea KI, Zerfas PM, Kastenmayer RJ, et al. Spontaneous pulmonary alveolar proteinosis in captive ‘moustached tamarins’ (*Saguinus mystax*) (Primates: Callitrichidae). Veterinary Pathology. 2012; 49:629–635. [PubMed: 21734058]

- Roux FJ, Lantuéjoul S, Brambilla E, Brambilla C. Mucinous cystadenoma of the lung. *Cancer*. 1995; 76:1540–1544. [PubMed: 8635055]
- Sato J, Kokoshima H, Tsuchitani M. Ovarian cystadenoma with smooth muscle proliferation in a cynomolgus monkey (*Macaca fascicularis*). *Veterinary Pathology*. 2008; 45:379–382. [PubMed: 18487498]
- Travis, WD.; Brambilla, E.; Muller-Hermelink, HK.; Harris, CC. World Health Organization Classification of Tumors. IARC Press; Lyon: 2004. Pathology and genetics of tumors of the lung, pleura, thymus and heart; p. 87

Fig 1.

Well-circumscribed, non-encapsulated, mucus-filled cystic mass located within the subpleural space, causing a nodular expansion and compressing the adjacent lung parenchyma with no evident invasive growth. Note a small area of extravasation of mucus in the periphery of the mass into normal lung parenchyma. HE. Bar, 1 mm.

Fig 2. Mucinous cystadenoma characterized by randomly-arranged cystic spaces lined mostly by a single layer of columnar to lower cuboidal mucus-secreting epithelial cells with occasional small papillary structures projecting into the lumen. HE. Bar, 100 μ m.

Fig 3. Mucinous cystadenoma. The mucus-producing cells and the eosinophilic material within the cysts are PAS positive. PAS with diastase digestion. Bar, 20 μ m.

Fig 4. Mucinous cystadenoma. A discrete and small area in the middle of the tumour labels for SP-B expression, suggesting trapped surfactant. IHC. Bar, 100 μ m.