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Case Report

Tracheal Stenosis and Adenocarcinoma in an Olive Baboon (*Papio cynocephalus anubis*)

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An adult female baboon (*Papio cynocephalus anubis*) presented for progressive difficulty in endotracheal intubation. Over a 7-y period prior to presentation, she was anesthetized and intubated 67 times for imaging by using single-photon emission computed tomography or positron emission tomography. Laryngoscopic examination revealed tracheal stenosis. Because of increased anesthetic risk and lack of alternative use, she was euthanized, and partial necropsy focusing on the larynx, trachea, and associated structures was performed. Gross examination revealed rigidity and functional fusion of the proximal 5 or 6 tracheal rings and narrowing of the lumen. Histology revealed ossification of tracheal rings and fibrosis of overlying tissue. In addition, a transmural umbilicated mass was present midway down the cervical trachea on its dorsolateral aspect. Histology of the tracheal mass identified a relatively well-circumscribed transmural adenocarcinoma. The combination of overall histologic pattern, evidence of anaplasia, and results of immunohistochemical staining was consistent with a diagnosis of adenoid cystic carcinoma. Anterior tracheal stenosis is a reported complication of intubation in humans and animals. Primary tracheal neoplasms are rare in domestic and research animals and, to our knowledge, have not previously been reported to occur in nonhuman primates.

Abbreviations: ACC, adenoid cystic carcinoma; HPF, high power field.

Case Report

History. An adult female olive baboon (*Papio cynocephalus anubis*) of unknown age, acquired in 2004 for evaluating nuclear imaging radiotracers, presented to the veterinary service for difficult intubation. Between March 2005 and February 2010, she had been anesthetized with intramuscular ketamine, intubated with a 4.5- or 5.0-mm endotracheal tube, and maintained with isoflurane for imaging by using single photon emission computed tomography or positron emission tomography on 67 occasions. For all but 1 of these 67 scans, she also was injected with a radiotracer as an intravenous bolus prior to scanning. There were 12 radiotracer targets, and all radiotracers were tagged with iodine-123. In 41 of the 67 scans, study drugs were administered, consisting of 15 different antidepressants and psychostimulants. All experimentation was performed under a protocol approved by the IACUC at Yale University. The baboon was seropositive for human papillomavirus 2 and *Cercopithecine herpesvirus* 2 (SA8); tested seronegative for *Herpesvirus papio* 2, measles, SIV, simian T-cell leukemia virus types 1 and 2, and simian virus SA11; and was negative for tuberculosis by intradermal skin test (and annual tuberculin immunoassay).

At the time of presentation, she vomited under anesthesia, and the endotracheal tube was removed. During attempts to reintubate, the endotracheal tube would not advance more than a few centimeters past the vocal folds.

Clinical and gross findings. Laryngoscopy performed a few weeks later revealed tracheal stenosis (Figure 1 A). Physical exam at that time was otherwise unremarkable. Because of increased anesthetic risk with this condition and lack of alternative use, the baboon was euthanized, and a partial necropsy that focused on the larynx, trachea, and associated structures was performed. Lymph nodes and other potential sites of metastasis were not examined due to the animal's radioactive status.

On gross examination, the first 5 or 6 tracheal rings distal to the larynx were firm, inflexible, and functionally fused. Longitudinal section of this region revealed approximately 30% narrowing of the lumen. Affected rings were very firm and had red-brown central discoloration suggestive of osseous metaplasia (Figure 1 B). A well-circumscribed mass (diameter, 2-cm) was located at the right dorsolateral aspect of the 13th and 14th tracheal rings (Figure 1 C). The mass penetrated to the mucosal surface, where it manifested as a flattened, 2-cm patch with an umbilicated center (Figure 1 D).

Histopathologic findings. After fixation in 10% formalin, tissues were embedded in paraffin, processed routinely, and blocks sectioned at 5 µm. Slides were stained with hematoxylin and eosin, periodic acid Schiff–Alcian blue, and Masson trichrome. Immunohistochemistry was performed on an automated system (Dako, Carpinteria, CA), using antibodies directed at human AE1–AE3 (general cytokeratin marker), CK7 (cytokeratin marker found in respiratory epithelium), p63 (marker of epithelial progenitor cells), Ki67 (proliferation marker), thyroglobulin (marker of thyroid epithelial cells), and CD117 (signaling molecule found in many tumor types).

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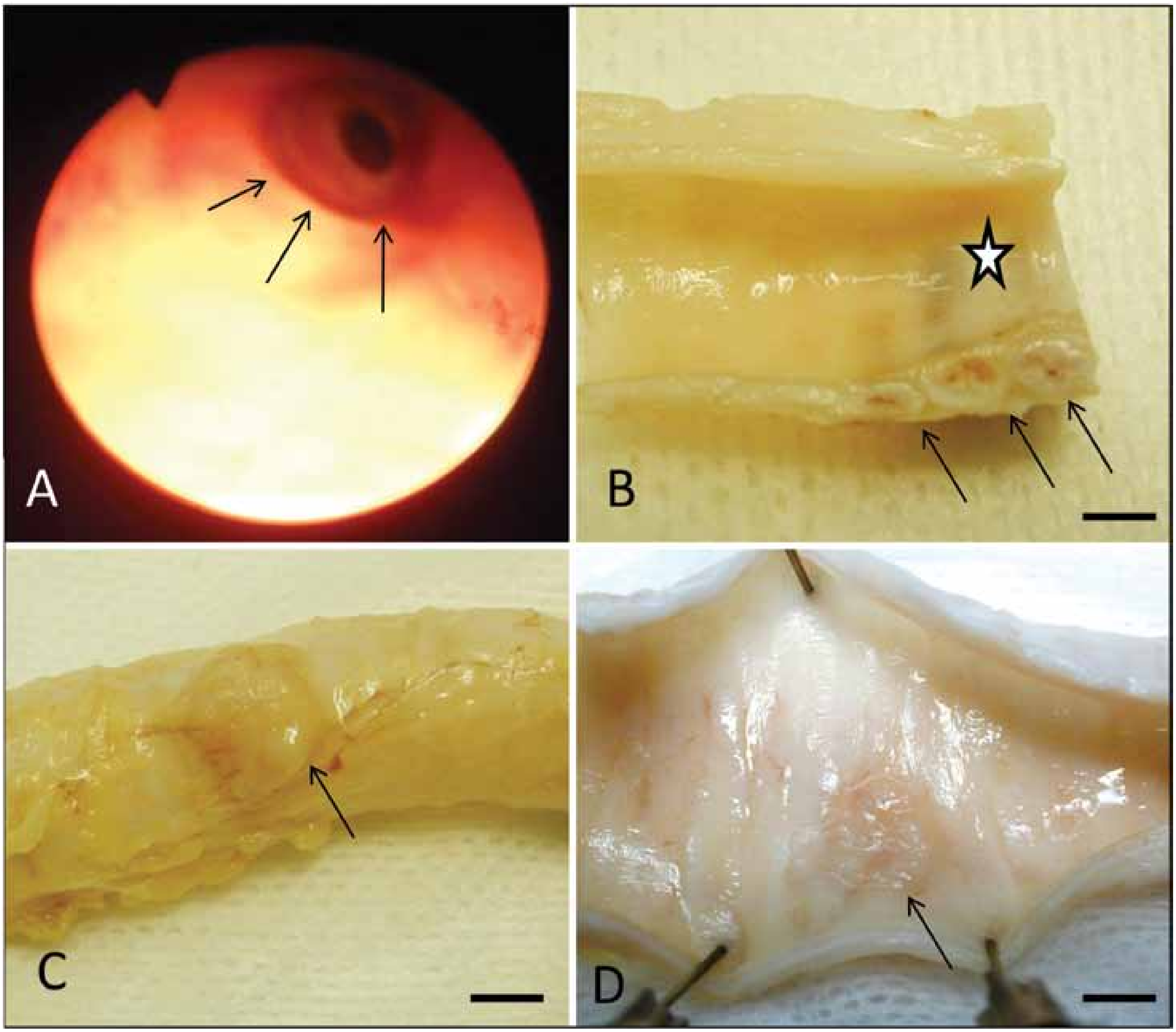

Figure 1. (A) Endoscopic view of tracheal stricture (arrows). (B) Longitudinal section of anterior trachea demonstrating stricture (asterisk) encompassed by thickened tracheal rings that contain bone marrow (arrows). (C) External view of the dorsolateral midtrachea, where a 2-cm tan mass is evident (arrow). (D) Luminal view of the tracheal mass (arrow). Bar, 1 cm.

Transverse sections of the stenotic region revealed osseous metaplasia of tracheal rings characterized by appearance of bone and bone marrow in the central region of each ring (Figure 2 A). The overlying mucosa was markedly thickened and fibrotic. This region was composed largely of fibrovascular tissue devoid of submucosal glands (Figure 2 C and D).

Histology of the tracheal mass identified a relatively well-circumscribed, multilocular adenocarcinoma that extended from the submucosa through the tracheal rings to form the grossly visible mass. Neoplastic tissue consisted of solid carcinomatous regions interspersed with cystic or tubular patterns (Figure 3 A). Tracheal lining epithelium could be seen descending from the lumen to connect with tubular and cystic or cribriform regions within the mass (Figure 3 B). Regardless of pattern, neoplastic cells rested on a clear basement membrane (Figure 3 C). These were lined by columnar, ciliated, or mucous epithelial cells and contained material that stained positive for periodic acid Schiff within their lumens. Epithelial cells in these regions were strongly positive for epithelial markers AE1–AE3 (not shown) and CK7 (a marker of respiratory epithelium, Figure 3 B) [27] and were enclosed by smooth muscle cells that were positive for smooth muscle actin, consistent with biphasic myoepithelial ductular differentiation (Figure 3 D). Immunoreactivity for these markers declined in solid areas, in favor of p63 (not shown), a marker of epithelial progenitor cells, and the proliferative marker Ki67 (Figure 3 E and F). Solid regions displayed cytologic evidence of dedifferentia-

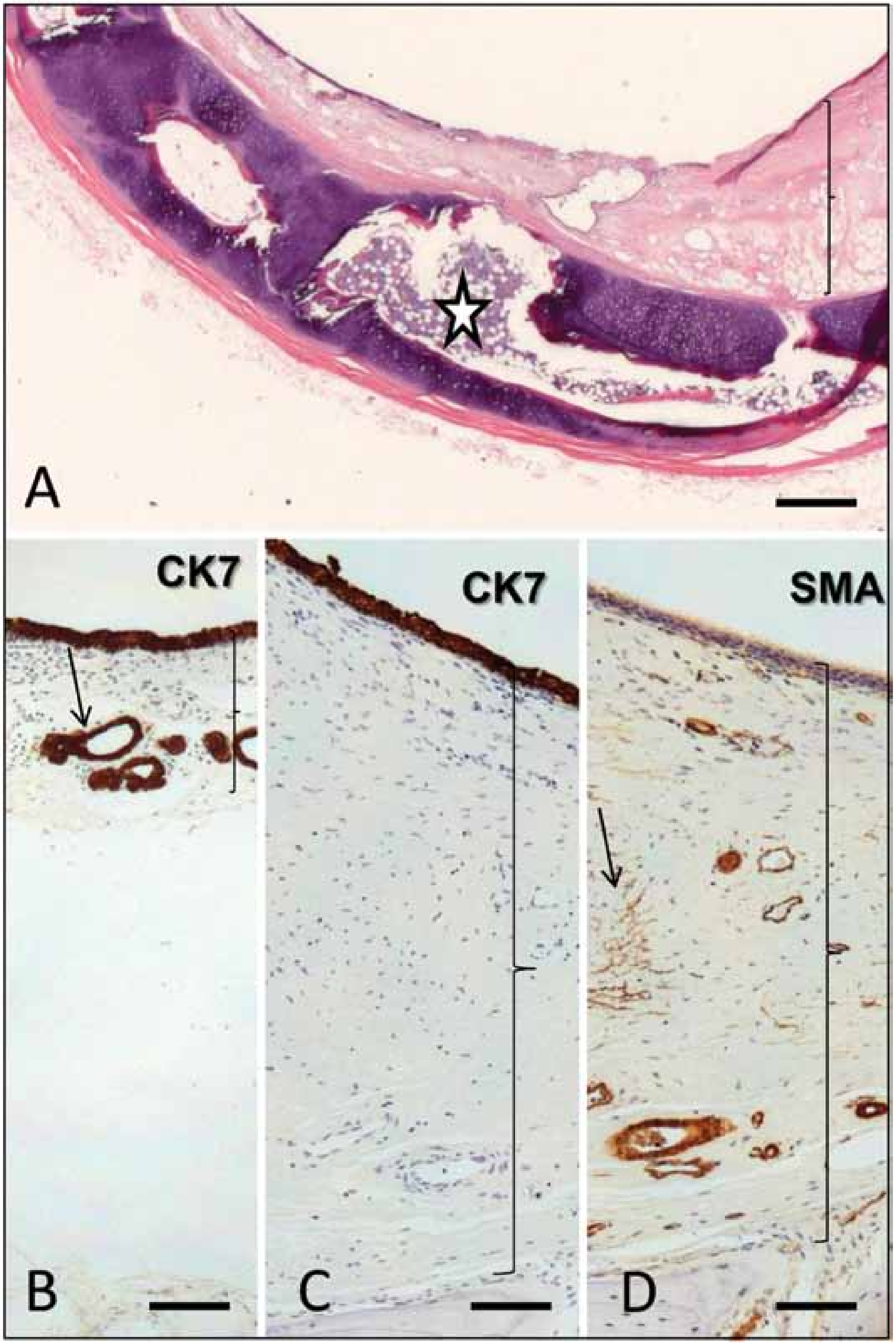

Figure 2. Narrowed tracheal lumen and distally located mass. (A) Low-power view of stenotic region in transverse section. Bone marrow is evident within tracheal rings (asterisk). Tracheal mucosa is markedly thickened and fibrotic (bracket). Bar, 200 µm. (B) CK7 immunohistochemistry, adjacent normal trachea. Lining and submucosal glandular epithelium epithelium is strongly immunoreactive (arrow). Width of tracheal submucosa is indicated with parentheses. Bar, 30 µm. (C) CK7 immunohistochemistry, stenotic trachea. Mucosa is markedly thickened (bracket), and submucosal glands have been lost. Bar, 30 µm. (D) Smooth-muscle actin immunohistochemistry, stenotic trachea. Numerous vascular profiles as well as diffuse nests of myoid tissue (arrows) are evident. Bar, 30 µm.

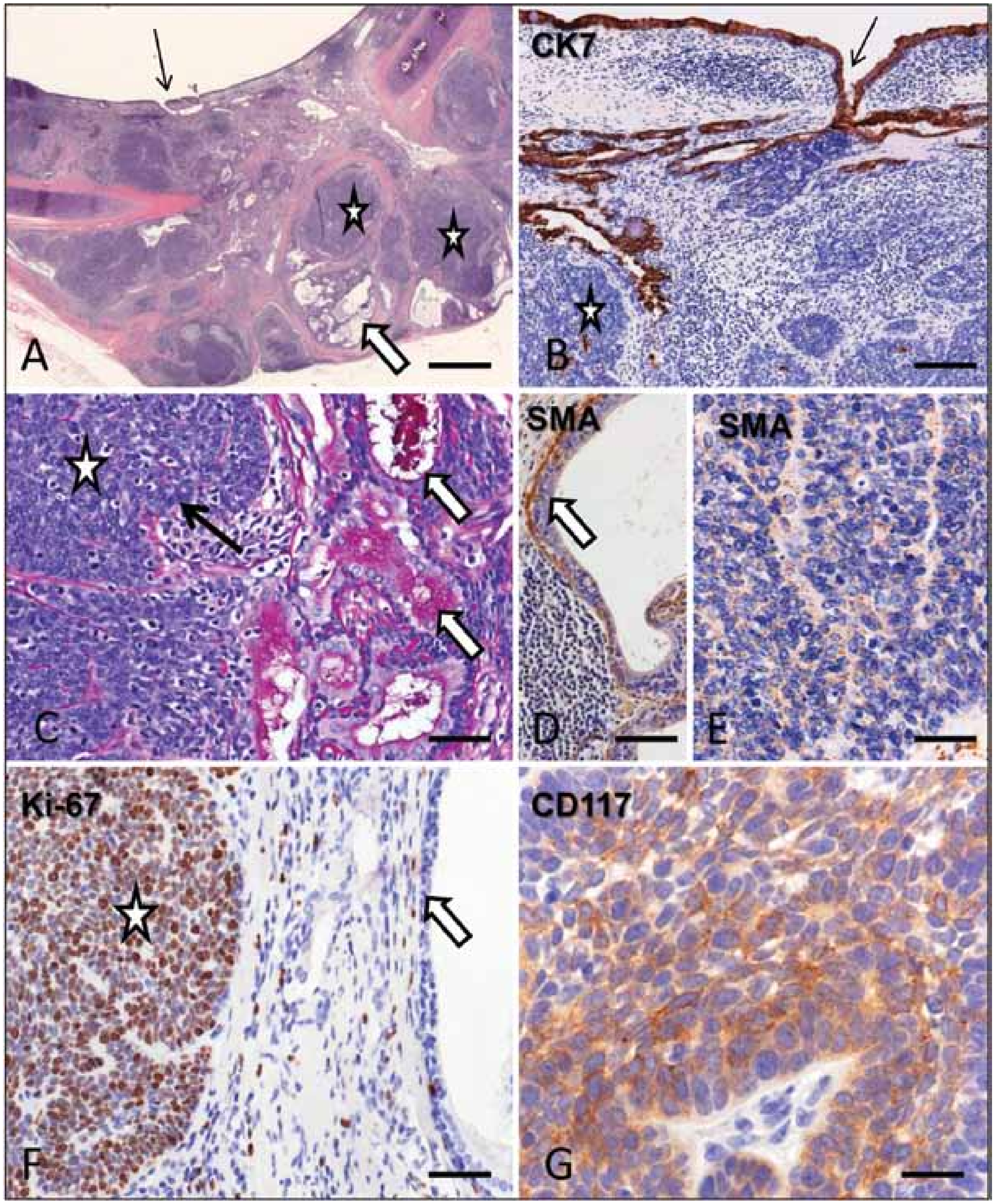

Figure 3. Histologic features of the tracheal tumor. (A) Low-power view of neoplastic tissue extending from the tracheal surface (arrow) through the tracheal rings. The tumor assumes a multiloculate appearance with alternating solid (asterisks) and cystic regions (white arrow). Hematoxylin and eosin stain; bar, 200 µm. (B) CK7 immunohistochemistry of tracheal surface and underlying tumor. CK7-positive epithelial cells are seen descending into the tumor to form trabeculae (arrow). CK7 immunoreactivity is diminished markedly in solid regions (asterisk). Bar, 50 µm. (C) High-power view of histologic patterns. Cystic regions are lined by columnar epithelium and contain immunopositive material (arrows). Basaloid cells arranged in sheets constitute less-differentiated solid regions (asterisk) that nevertheless still rest on a basement membrane. Periodic acid Schiff stain; bar, 30 µm. (D, E) Smooth-muscle actin immunohistochemistry. Strong bilaminar immunoreactivity is noted in regions of ductular differentiation (arrow, D). Weaker, more diffuse immunoreactivity prevails in solid areas (E) Bar, 30 µm. (F) Ki67 immunoreactivity. Rare immunopositive nuclei are evident in cystic regions (arrow), whereas diffuse immunoreactivity is present in solid areas (asterisk). Bar, 30 µm. (G) CD117 immunoreactivity. Immunoreactivity with a membrane-staining pattern is present in solid regions. Bar, 20 µm.

tion. Tumor cells in these regions were round to spindeloid, with moderate cellular atypia (anisocytosis and anisokaryosis), a mitotic rate of approximately 4 mitoses per 10 high-power fields and frequent apoptotic bodies. Cells abutting basement membrane were arranged in crude palisades; however, this pattern was lost toward the interior of each nodule. Multifocal immunoreactivity against CD117 (alternatively known as the c-Kit receptor, a cytokine receptor expressed on hematopoeitic cells as well as several subtypes of salivary gland tumors) was detected predominantly in solid areas (Figure 3 G). Thyroglobulin immunohistochemistry was negative. The combination of the overall histologic pattern (solid, tubular, and cribriform regions enclosing material positive for periodic acid Schiff), evidence of anaplasia and proliferative vigor in solid regions, and CD117 immunoreactivity is consistent with diagnosis of adenoid cystic carcinoma.[2,8,23,26,28]

Discussion

We suspect that the tracheal stenosis and adenocarcinoma were unrelated findings. The tracheal stenosis likely was due to repeated or traumatic endotracheal intubation. The tracheal adenocarcinoma may or may not be due to experimental intervention, but the lesion is unlikely due to endotracheal intubation because of the distal location of the mass.

Complications due to tracheal intubation occur commonly in humans.[7,17,29] These complications first emerged historically during mechanical ventilation required by poliomyelitis. Sequelae of prolonged tracheal intubation can include laryngeal damage, subglottic stenosis, and tracheal stenosis. Despite improvements in endotracheal tubes, tracheal stenosis after intubation remains an important cause of tracheal obstruction.[17,29] Research studies of stenosis at the cuff site have shown that the main cause is the pressure exerted on the tracheal mucosa by the tube cuff.[7] A cuff pressure greater than 30 mm Hg exceeds the mucosal capillary perfusion pressure, causing mucosal ischemia, which can lead to ulceration and chondritis of the tracheal cartilages.[18] These circumferential lesions heal by fibrosis, leading to a progressive tracheal stenosis.[29]

Tracheal stenosis secondary to cartilage ossification without trauma due to intubation has also been reported. Tracheal cartilage typically does not ossify, but one study found ossification of the tracheal cartilage in 52% of adults (age, 20 to 87 y).[19] Tracheal injury secondary to intubation has been described in domestic and research animals including, but not limited to, dogs, cats, horses, and rabbits. Injuries include: tracheal stricture formation, tracheal rupture, tracheal ulceration, and respiratory obstruction by necrotic tracheal debris.[3,14,15,22,24]

Although we found no reports of tracheal stenosis in nonhuman primates in the literature, factors contributing to tracheal stenosis in humans and other domestic and research animals are also likely apply to nonhuman primates. In particular, repeated intubation of laboratory animals for research purposes may contribute to stenosis formation. However, repeated intubations may be necessary to comply with the 3Rs, especially reduction in the number of animals used.

In humans, primary neoplasia of the trachea is rare, accounting for less than 0.5% of all tumors.[1,20,25] Tumors may arise from tracheal epithelium, tracheal glands, or mesenchymal structures. Adenoid cystic or squamous cell carcinoma account for about two-thirds of adult human primary tracheal tumors, with the remaining third distributed across a wide range of epithelial and mesenchymal tumor types.[1,2,11-13] Adenoid cystic carcinoma is a malignant tumor that must be differentiated from other myoepithelial tumors, particularly the less aggressive but histologically similar variant known as polymorphous low-grade adenocarcinoma.[2,8,26,28] According to Bosman et al., 2010, the adenoid cystic carcinoma in the baboon we present conformed to the features used in the World Health Organization (WHO) classification system for tumors in humans.[4] The incidence of adenoid cystic carcinoma is the same in both sexes and is most common in patients in the fourth and fifth decades of life. Low-grade adenoid cystic carcinoma progresses slowly and has a propensity to spread along both mucosal and perineural planes. Only 10% of patients have regional lymph node or remote metastases.[12,13] The predominance of dedifferentiated solid regions in our animal's tumor supports its characterization as a high-grade lesion and suggests aggressive tumor behavior.[23,25]

Although adenocarcinoma is a relatively common spontaneous neoplasm in nonhuman primates, tracheal neoplasia is extremely uncommon. In a review of spontaneous neoplasia in baboons,[5] adenocarcinoma was the second most common neoplasm (lymphosarcoma was most common). Tumor locations included duodenum, cecum, gall bladder, papilla of Vater, pancreas, and ovarian surface. Of 204 published cases of spontaneous neoplasia in *Papio* species, only one animal was found to have tracheal, in addition to vaginal, papillomas.[5] Given that we performed only a partial necropsy in our baboon, whether tumor was located in any organ other than the trachea is unknown.

Primary tracheal neoplasms are rare in domestic and research animals; as of 2003, only 37 cases have been reported in the veterinary literature.[4] Affected animals include dogs, cats, pigs, and pythons.[4,9,10] Both benign and malignant tumor types occur. Primary tracheal tumors reported in dogs include adenocarcinoma, carcinoma, mast cell tumor, leiomyoma, extramedullary plasmacytoma, osteosarcoma, chondroma, chondrosarcoma, osteochondroma, and ecchondroma. Primary tracheal tumors in cats have included lymphoma, adenocarcinoma, seromucinous carcinoma, adenoma, and squamous cell carcinoma.[4,14] The WHO International Classification of Tumors of Domestic Animals defines adenoid cystic carcinoma arising from salivary glands or submucosal glands of the respiratory system by using histologic criteria similar to those for humans. This lesion is considered rare and has not previously been reported in the trachea of animals. Myoepithelial head and neck tumor variants reported in humans are too infrequently reported in animals to ascribe prognostic data with individual tumor types in animals.

Carcinomas can develop in areas associated with prolonged irritation or mechanical trauma, chronic infection, or atmospheric carcinogens. In the case we present here, repeated trauma of intubation may have caused or contributed to tracheal stenosis; however, the tumor was too distal to have been caused by intubation. In addition, transformation of tracheal papillomas into carcinomas after isotope or X-ray therapy of thyroid tissue has been reported to occur in humans.[21] Possible radioactive damage or high concentration of targeted receptors within the respiratory epithelium theoretically could account for the development of tracheal adenocarcinoma.[16] ^{123}I (sodium iodide), the radioactive tracer used in the baboon we present, is used commonly during nuclear imaging. ^{123}I compares equally with ^{131}I for uptakes studies but significantly reduces thyroid radiation exposure, in that ^{123}I is not as carcinogenic as ^{131}I.[16] No long-term animal studies have been performed to evaluate the carcinogenic potential of ^{123}I in veterinary patients. In addition, the tumor in question was thyroglobuliun-negative, and the mass was 30 cm distal to the thyroid gland, making ^{123}I radiation exposure an unlikely cause of our baboon's lesion. To our knowledge, tracheal adenocarcinoma has not previously been reported to occur in nonhuman primates.

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