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Spontaneous Primary Squamous Cell Carcinoma of the Lung in a Rhesus Macaque (*Macaca mulatta*)

Sherrie M Jean,[1] Pablo R Morales,[2] Katherine Paul,[3] and AnaPatricia Garcia[4,5,*]

A 3-y-old male rhesus macaque (*Macaca mulatta*) was noticed to be lethargic in the compound. Physical exam revealed cyanotic mucous membranes, dyspnea, bilateral harsh lung sounds, wheezing on expiration, and a firm mass possibly associated with the liver. Radiographs revealed bilateral soft tissue opacities in the thorax. Due to poor prognosis, the rhesus was euthanized, and a necropsy was performed. Both right and left lung lobes were consolidated and had multifocal white–tan masses. On cut section, the masses were firm, had areas of necrosis, hemorrhage, and often contained a tenacious exudate. Masses were identified in the liver and both kidneys. Given the morphologic features of the neoplasm, a diagnosis of squamous cell carcinoma was made. Immunohistochemistry staining for thyroid transcription factor, a nuclear transcription factor normally found in lung, thyroid, and tumors arising from either of those tissues, confirmed that the masses originated from the lung. Malignant primary lung tumors are divided into 8 main histologic subtypes: squamous cell carcinoma, small-cell carcinoma, large-cell carcinoma, adenocarcinoma, adenosquamous carcinoma, sarcomatoid carcinoma, carcinoid tumor, and salivary gland tumors. Clinical signs associated with lung tumors include, but are not limited to, dyspnea, coughing, hemoptysis, lethargy, anorexia, and weight loss. Although squamous cell carcinoma will be low on the differential list for these clinical signs, we encourage clinicians and researchers to not rule it out solely based on incidence and age of the animal.

Abbreviations: SCC, squamous cell carcinoma; TTF1, thyroid transcription factor 1.

Primary lung tumors are rare in nonhuman primates. To date, only 2 publications have documented spontaneous primary pulmonary squamous cell carcinoma (SCC) in nonhuman primates.[5,11] One report described a case of primary pulmonary SCC in a 7-y-old cynomolgus macaque (*Macaca fascicularis*).[11] The other report documented 3 cases of spontaneous pulmonary squamous cell carcinoma in both *Tupaia belangeri* and *Saguinus fuscicollis* that occurred during 1978 to 1994.[5] SCC has been documented to occur in rhesus macaques (*Macaca mulatta*) but never as a primary lung tumor. Other primary pulmonary neoplasias in nonhuman primates have included bronchioloalveolar adenoma, bronchial adenoma, bronchiogenic carcinoma, carcinoid, clear cell carcinoma, and small cell carcinoma.[5,11]

In 2004, the World Health Organization published its revised classification scheme for malignant lung and pleural tumors. This scheme divides malignant lung tumors into 8 main categories: SCC, small cell carcinoma, large cell carcinoma, adenocarcinoma, adenosquamous carcinoma, sarcomatoid carcinoma, carcinoid tumor, and salivary gland tumors.[3] In humans, lung cancer is the leading cause of death from malignant tumors in the United States, and the major risk factor for development is smoking.[2,3,6,7,10,17,20] Other risk factors in humans include exposure of the airways to carcinogens such as asbestos, arsenic, radon, chromium, nickel, and radiation, and previous pulmonary fibrosis due to chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, or tuberculosis.[2,7,17] A study of human lung carcinoma found that the incidence of SCC was low in patients younger than 30 y; the authors further speculated that unidentified environmental factors rather than smoking and genetic factors likely played a more important role in young patients.[15] Given that most nonhuman primates are not routinely exposed to tobacco smoke, this same hypothesis is likely true with regard to the predisposing factors for primary lung tumors in young nonhuman primates as well.

The present report describes a case of primary SCC of the lung, confirmed with immunohistochemistry and electron microscopy, that metastasized to the liver and kidneys in a 3-y-old rhesus macaque (*Macaca mulatta*). We also present a brief overview of the different categories of lung tumors and their characteristics according to the World Health Organization classification scheme.

Case Report

The 3-y-old male rhesus macaque (weight, 4.8 kg) in this report was housed at the Yerkes National Primate Research Center (Atlanta, GA), an AAALAC-accredited institution. All research and animal care at this facility was performed in accordance with the *Guide for the Care and Use of Laboratory Animals* and the *Animal Welfare Act*.[1,9] Any and all research performed was approved by the Institutional Animal Care and Use Committee. The macaque was fed a standard diet of monkey chow (Purina Mills Lab Diet no. 5037, PMI Nutrition International, St Louis, MO) twice daily and county municipal tap water ad libitum. Daily the monkey was given oranges and an enrichment item consisting of an alternating schedule of edible and destructible items.

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[1]Department of Animal Resources, University of Southern California, Los Angeles, California; [2]The Mannheimer Foundation, Homestead, Florida; [3]Centers for Disease Control and Prevention, Atlanta, Georgia; [4]Division of Pathology, Yerkes National Primate Research Center and [5]Department of Pathology and Laboratory Medicine, School of Medicine, Emory University, Atlanta, Georgia.
[*]Corresponding author. Email: agarci5@emory.edu

The macaque was housed in an outdoor breeding colony while assigned to a behavioral development study and was admitted to the veterinary hospital for extreme lethargy after an aggressive, traumatic encounter with conspecifics in the compound. During cageside observation, the macaque was alert and responsive but lethargic and appeared to have difficulty breathing. The macaque was anesthetized with ketamine hydrochloride (10 mg/kg; Ketaset, 100 mg/mL, Fort Dodge Animal Health, Fort Dodge, IA) for physical examination. Physical exam findings consisted of cyanotic mucous membranes, tachypnea, and dyspnea with bilateral harsh lung sounds and wheezing on expiration. Cardiac sounds could not be auscultated due to the obstructive lung noise. The liver was enlarged on abdominal palpation, and there was a firm mass midabdominally that seemed to be associated with the liver. Radiographs of the thorax and abdomen showed evidence of bilaterally increased soft-tissue opacity in the thorax; the opacity was considerably more pronounced on the right (Figure 1). At the time of exam, the leading differential diagnosis was diaphragmatic hernia. Given its poor prognosis and unlikely recovery and return to the colony, the macaque was euthanized.

Gross necropsy findings. On entering the abdomen, the linea alba was widened markedly and prominent. Adhesions extending from the liver to the abdominal wall and diaphragm were present. The diaphragm was intact, and there was no evidence of a current or previous diaphragmatic hernia. Both left and right lung lobes were adherent to the pleura of the thorax, pericardial sac, trachea, and esophagus. The pericardial sac was thickened, and the heart was displaced to the left side of the thorax. Both lungs were consolidated and had multifocal white to tan masses. The proximal right lung lobe had a 12.0 × 10.0 cm dark mass that on cut section was cavitated and contained tenacious exudates (Figure 2). The distal lung lobe had multifocal tan to red raised nodules that often exhibited umbilicated centers. These masses ranged from 1.0 to 7.0 cm in diameter. The proximal lobe of the left lung had a small raised nodule that was 0.5 cm in diameter. The distal lobe was almost completely replaced by a dark red firm mass that measured 12.0 × 12.0 cm. On cut section, the masses were firm had areas of necrosis and hemorrhage and often contained a tenacious exudate. The left hepatic lobule had a raised dark red to tan mass that measured 5.0 cm in diameter (Figure 3) and that on cut section contained a similar tenacious exudates as did the lung nodule. The caudate and right hepatic lobes had multiple tan to red raised nodules ranging in diameter from 0.3 to 3.0 cm. On cut section, these nodules had areas of necrosis and hemorrhage. The left kidney had 2 white foci that measured 0.2 cm in diameter. The right kidney had a raised white nodule that measured 0.5 cm in diameter and on cut section had a similar exudate to that of other cut nodules. Both kidneys were pale. Specimens from the masses, heart blood, lung, and liver were obtained for bacterial culture. Sections of masses and major organs were collected and fixed in 10% neutral buffered formalin. Tissue samples were embedded in paraffin, sectioned, and processed for routine histologic evaluation.

Histologic findings. The lung contained large multifocal areas of necrosis intermixed with large lakes of eosinophilic material with cords, sheets, and nests of round to oval neoplastic cells. These cells had large vesicular round to oval nuclei with 1 or 2 prominent nucleoli and abundant eosinophilic cytoplasm (Figure 4). Occasional neoplastic cells exhibited marked eosinophilia of the cytoplasm as well as marked anisocytosis and anisokaryosis. Bizarre multinucleated giant cells were observed often. Mitotic figures were common and present at a rate of 1 to 2 per high-power field. Scattered in the less affected pulmonary

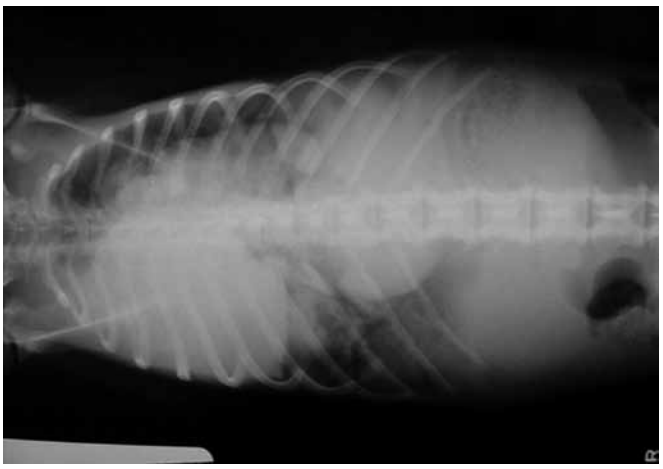

Figure 1. Ventrodorsal thoracic radiograph taken of macaque, displaying bilateral intrathoracic soft tissue opacities. Note the lesion is more extensive and severe on the right than the left.

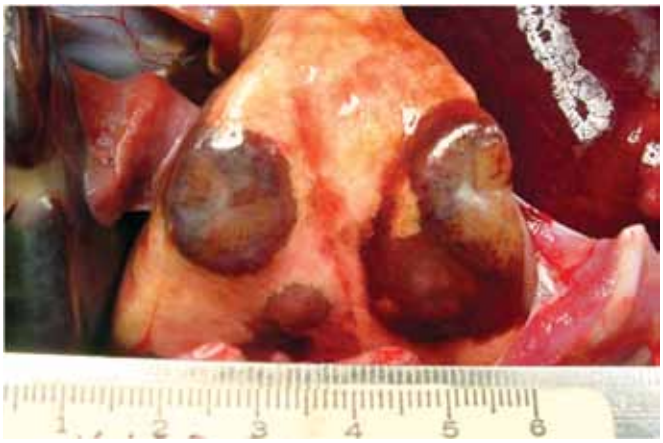

Figure 2. Photograph of the open thoracic cavity of the rhesus monkey (cranial to the left). (A) Right lung with multifocal, raised, umbilicated masses.

parenchyma were areas of edema intermixed with areas of moderate to severe infiltration of neutrophils and hemosiderin-laden macrophages. The bronchi contained large numbers of neutrophils.

Histologically, the liver had multifocal areas of proliferation of similar round to oval neoplastic cells surrounding a central area of necrosis. The neoplastic cells were arranged in sheets and nests and often had vesicular nuclei and abundant eosinophilic cytoplasm. Mitotic figures were common (1 or 2 per high-power field).

The right kidney had a large focal area of necrosis admixed with cords and islands of similar neoplastic cells. Rare blood vessels contained small aggregates of neoplastic epithelial cells. No other significant lesions were observed in other organs examined.

Based on the morphologic features of the neoplasm, a diagnosis of carcinoma was made. To further characterize this tumor, ancillary testing including electron microscopic evaluation and immunohistochemistry was performed. Electron microscopic evaluation of the pulmonary neoplastic cells revealed variable numbers of desmosomes (Figure 5) and many tonofilaments (Figure 6). In addition, immunohistochemistry of the lung and liver masses was performed. Neoplastic cells were negative for vimentin at both sites (liver and lung). Immunoreactivity for cytokeratin was positive in the pulmonary and hepatic neoplastic

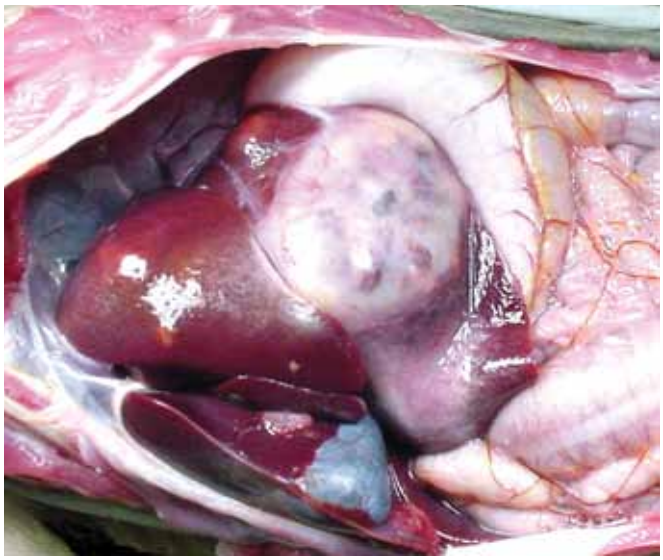

Figure 3. Photograph of the liver from this monkey, depicting a large, raised, tan-colored neoplasm roughly in the center of the photograph.

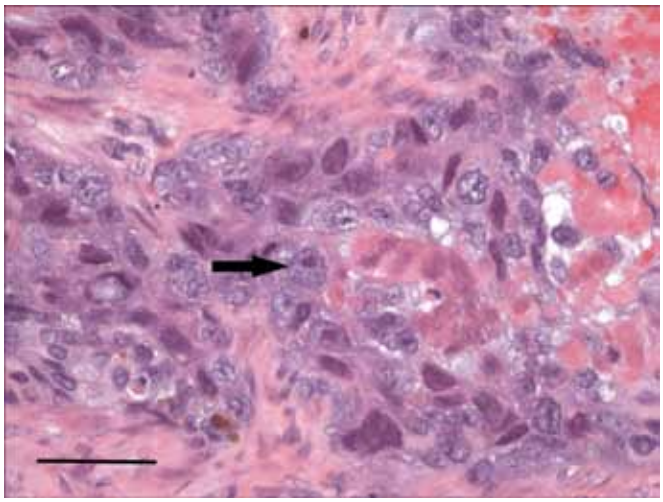

Figure 4. Photomicrograph of the neoplastic cells in the lung. Note the prominent nucleoli and abundant eosinophilic cytoplasm. Bar, 210 µm

masses. To confirm that the primary origin of the tumor was lung, we stained for thyroid transcription factor 1, a nuclear transcription factor normally found in lung, thyroid, and tumors arising from either of those tissues.[4,8] Tumor metastases to the lung from other primary sites are typically negative for this protein. Immunoreactivity for thyroid transcription factor 1 was positive in neoplastic cells of sections of the liver (Figure 7).

Discussion

Primary lung tumors in nonhuman primates are rare.[5,11] Even more rare are primary tumors in young and young adult animals. A study done at the German Primate Center during 1978 to 1994 documented an incidence rate of 1.08% among 54 tree shrews and 409 adult callitrichids. That study[5] documented 11 pulmonary tumors, 3 of which were squamous cell carcinoma. The current report presents a unique case of primary pulmonary squamous cell carcinoma in a 3-y-old male rhesus macaque that was housed in an outdoor colony and had not received or participated in any experimental procedures.

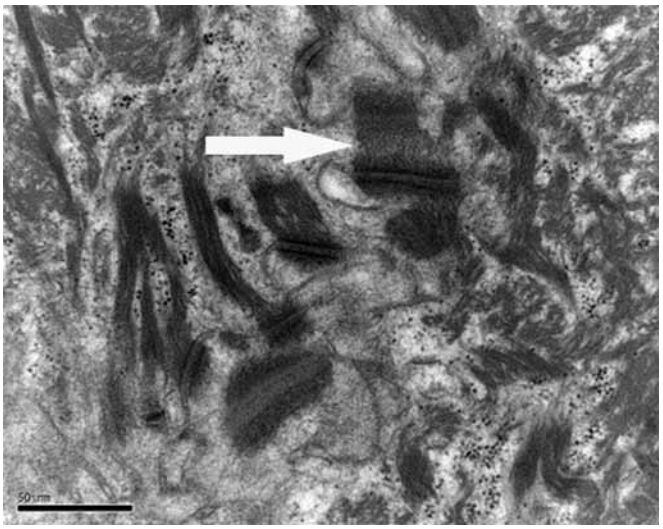

Figure 5. Electron micrograph displaying the typical desmosomes (arrow) observed in the neoplastic cells.

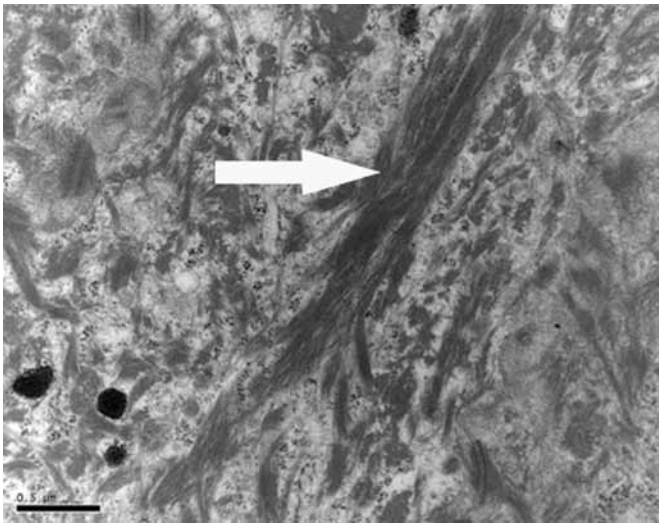

Figure 6. Electron micrograph of the tonofilaments (arrow) present in cells of the lung tumor.

The World Health Organization updated and published a revised classification scheme for primary lung tumors in humans that details the characteristics of benign lung tumors, preinvasive lung lesions, and malignant primary tumors.[3,21] Malignant primary tumors are broken down into 8 main histologic subtypes: squamous cell carcinoma, small cell carcinoma, large cell carcinoma, adenocarcinoma, adenosquamous carcinoma, sarcomatoid carcinoma, carcinoid tumor, and salivary gland tumors.[3] The World Health Organization classification scheme also recognizes 3 precancer lesions that can lead to the development of metastatic cancer: squamous dysplasia/carcinoma in situ, atypical adenomatous hyperplasia, and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia. The progression from precancer states to cancerous lesions is well established. Lesions usually progress from epithelial cell hyperplasia to squamous metaplasia typically with dysplasia and then to carcinoma in situ before finally becoming neoplastic.[3,6,17,20] According to the described classification system, these primary malignant lung tumors need to be included in the differential diagnosis when evaluating a lung neoplasm.[3]

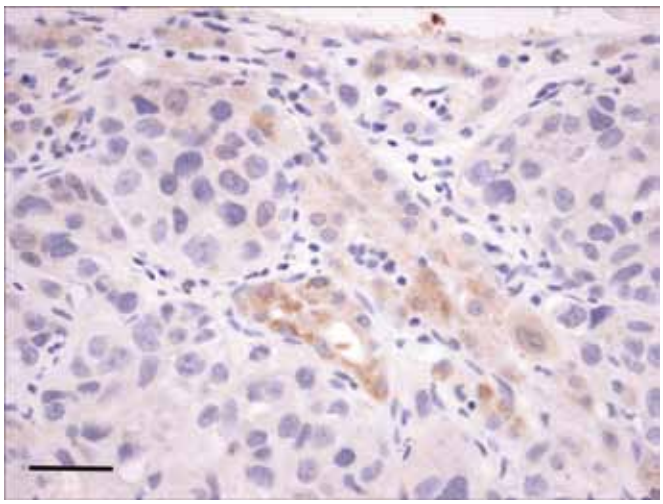

Figure 7. Photomicrograph of a positive immunohistochemistry stain using antithyroid transcription factor to confirm the neoplastic cells' primary (pulmonary) origin.

In humans the most common type of primary lung tumor is squamous cell carcinoma (SCC).[13,20] These tumors have been reported in 30% of all lung cancer patients.[21] SCC typically present as central lung tumors most often arising in the segmental bronchi with extension into the lobar and mainstem bronchus, but a small percentage of them arise in the periphery of the lung.[21] SCC typically do not metastasize until late in the course of the disease.[7] Some characteristics of SCC include intercellular bridging, individual cell keratinization, and laminated squamous 'pearl' formation.[13,20] Ultrastructurally, SCC contain desmosomes and tonofilaments. In our case, electron microscopic evaluation of the primary tumor demonstrated the presence of desmosomes and tonofilaments.

The major risk factor in the development of SCC in humans is cigarette smoking.[10,18] In addition to smoking, exposure to asbestos, polycyclic aromatic hydrocarbons, arsenic, and chromium compounds, radiation, and other occupational agents undoubtedly account for some of these cases. Furthermore, vitamin A and folate deficiencies contribute to the development of multifocal squamous metaplasia, which in turn can predispose tissue to becoming neoplastic.[13] All of the nonhuman primates at our facility are fed the appropriate nutritionally balanced standard monkey chow and therefore are unlikely to have vitamin and mineral deficiencies.

In macaques, only 2 case reports of SCC of the lung have been reported, with only 1 of the lesions originating in the lungs—a subpleural tumor found incidentally at necropsy in a cynomolgus macaque.[11] That animal had been involved in a chronic toxicity study, the details of which were not described. Whether the toxicity study predisposed the macaque to develop SCC was not discussed either. The macaque was not described to have exhibited any clinical signs in association with this tumor prior to necropsy. No metastasis from the tumor or blood vessel invasion was observed.[11] Although there was no evidence of other tumor sites, ancillary tests were not done to confirm the primary site of the tumor. There was also mention of lung mite, *Pneumonyssus simicola*, pigment in the lung, indicative of a previous infestation.[11] This event alone or in combination with the toxicity experiments could have predisposed the reported macaque to develop squamous dysplasia or metaplasia and thus neoplasia.

In our case, the rhesus macaque was not involved in any experimental procedures, was not intentionally exposed to any known carcinogens, had no evidence of a previous or ongoing lung mite infestation, and had no evidence of a previous underlying chronic infectious or inflammatory process such as chronic obstructive pulmonary disease or tuberculosis. The nonhuman primates at our facility are tested annually by tuberculin skin testing for tuberculosis; the reported macaque had no record of a positive test. Because our macaque was housed in an outdoor breeding colony, exposure to environmental agents or carcinogens cannot be ruled out entirely, nor can a genetic predisposition. Although several reports have attributed environmental air pollutants as risk factors for the development of lung cancer, they are not considered a major risk factor.[12,19] At our facility, all nonhuman primates that die or are euthanized undergo a complete postmortem examination; the reported animal was the only one with pulmonary SCC at our facility. The monkey in the current report also differs from the previously reported case in a cynomolgus macaque[9] because our macaque had a more advanced stage of cancer and exhibited pronounced clinical signs as a result of the lung lesion; furthermore, corresponding evidence of pulmonary pathology was evident on radiographs. Numerous metastases were found in the liver and kidney of our animal grossly and microscopically. Because of the location of these metastases, immunohistochemistry was performed to confirm that the tumors were of primary pulmonary origin. Thyroid transcription factor 1 (TTF1) is expressed in the thyroid gland, diencephalon, and bronchioalveolar epithelium. In the thyroid gland, TTF1 regulates the expression of thyroid peroxidase and thyroglobulin.[14,16] In the lung, TTF1 is involved in the regulation of surfactant protein production. In the adult lung, TTF1 is expressed in the noncilliated bronchiolar epithelial cells and in type II cells. The protein plays a role in epithelial morphogenesis, stimulates the synthesis of pneumocyte surfactant proteins, and regulates secretory product gene transcription in Clara cells.[14,16] Therefore, its detection in metastases in brain or other sites is an almost certain indicator that the primary tumor arose from lung. In our monkey, neoplastic cells from the liver were positive for TTF1 protein, confirming the pulmonary origin of the neoplasm.

Clinical signs associated with lung tumors include, but are not limited to, dyspnea, coughing, hemoptysis, lethargy, anorexia, and weight loss. The macaque in the current case report showed signs of dyspnea and lethargy, but anorexia, weight loss, and coughing were not observed despite the aggressive and advanced stage of disease. It seems unlikely that the animal was exposed to any of the previously mentioned known risk factors for pulmonary neoplasia in humans. If a patient is exhibiting signs of dyspnea, coughing, lethargy, and so forth and pulmonary disease is suspected, several diagnostic techniques can be used to help move neoplasia up or down on the differential list. Radiology, ultrasonography, thoracocentesis, excisional biopsy of an accessible lymph node, sputum cytology, flexible bronchoscopy, transthoracic needle aspiration, and computed tomography are some of the less-invasive techniques.[7] Thoracotomy is the most invasive and usually the last resort. Although invasive, thoracotomies can be diagnostic as well as curative if there is a single peripheral mass that can be excised in its entirety with sufficient margins.[7] At this time, this type of intervention is not routinely done in laboratory animal medicine.

Although the incidence of SCC is likely too low to elucidate its primary risk factors and prognosis in nonhuman primates, the techniques used for diagnosis appear to be consistent with those used in other species. Although SCC will be low on the differential list, we encourage clinicians and researchers to not rule it out solely based on incidence and the age of the animal. We recommend that, when pulmonary neoplasia with metastasis

is encountered, pathologists perform immunohistochemistry for TTF1 on neoplastic tissues to identify the primary origin of the neoplastic cells.

Acknowledgments

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