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Zoo Animals

Imaging and Pathological Comprehensive Analysis of Oesophageal Squamous Cell Carcinoma in a Rhesus Monkey (*Macaca mulatta*)

Heling Li[1,2] | Lixian Chen[3] | Long Zhang[3] | Xinglong Chen[1] | Hong Wang[1,2]

[1]State Key Laboratory of Primate Biomedical Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, Yunnan, China | [2]Yunnan Key Laboratory of Primate Biomedical Research, Kunming, Yunnan, China | [3]Kunming Biomed International Ltd., Kunming, Yunnan, China

Correspondence: Hong Wang (wangh@lpbr.cn)

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ABSTRACT

Squamous cell carcinoma (SCC) has a high incidence in non-human primates (NHPs), which affects animal health and causes huge losses to the experimental monkey breeding industry. In this case, gastroscope showed a smooth-surfaced elevated lesion located approximately 18 cm from the incisor teeth of the diseased rhesus monkey. Computed tomography (CT) showed a space-occupying lesion in the oesophagus. Haematoxylin & eosin (HE) staining revealed an invasive tumour of polygonal nests in the submucosa and muscularis, while keratosis and nuclear atypia were also observed. Immunohistochemistry (IHC) studies revealed tumour cells positive for p63, CK5/6, p40, CK20, CK7 and CK19. Based on imaging and pathology findings, the rhesus monkey was diagnosed as oesophageal squamous cell carcinoma (ESCC) and was treated surgically. Unfortunately, the rhesus monkey was euthanized eventually according to its poor health condition. This provides an important reference for the diagnosis and treatment of ESCC in rhesus monkeys, and also provides an important spontaneous animal model for future research on ESCC.

1 | Introduction

Non-human primates (NHPs) are ideal animal models for basic and preclinical research to solve human disease problems. However, the emergence of various spontaneous diseases in rhesus monkeys presents significant challenges to veterinary diagnosis and treatment, as these diseases not only disrupt experimental results but also affect the overall research process. Squamous cell carcinoma (SCC) is an epithelial malignant tumour that can originate from various areas in the body including the tongue, oesophagus and throat (Aoki et al. 2023). SCC is a common spontaneous malignant tumour in NHPs, including cynomolgus monkey (*Macaca fascicularis*) (Nakamura et al. 2000), rhesus monkey (Jean et al. 2011), capuchin monkey (Grana et al. 1992), sooty mangabey (Morales et al. 2006), baboon and spider monkey (Haddad et al. 2009). In this study, we used imaging methods including gastroscopy and CT, Haematoxylin & eosin (HE) staining and extensive IHC for pathological examination and surgical treatment of the monkey. This study provides an important reference for the diagnosis and treatment of ESCC in rhesus monkeys and also provides an important spontaneous animal model for future research on ESCC.

Heling Li and Lixian Chen contributed equally to this work.

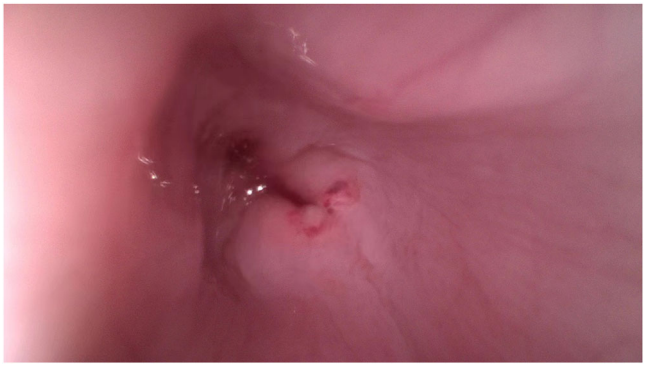

FIGURE 1 | Gastroscope examination showed hyperplasia of oesophageal tissue.

2 | Case Presentation

A 14-year-old female rhesus monkey weighing 3.4 kg, from the State Key Laboratory of Primate Biomedical Research, Kunming University of Science and Technology [SYXK (Yunnan) K2022-0001], was examined. It was housed in a single cage with a temperature-controlled chamber with free access to water and food. The monkey displayed frequent vomiting, sunken eyes, poor skin elasticity, progressive wasting and serous nasal discharge. Physical examination of the animal showed a respiration rate of 28 times/min, unsedated exam heart rate of 198 beats/min and capillary refill time (CRT) < 2 s. Venous blood was collected for complete blood count, blood biochemistry and serum electrolytes. The complete blood count showed a white blood cell count of 15.4 × 10^9/L (reference value: 6.0–14.0 × 10^9/L) and a haematocrit level of 52.4% (reference value: 35.0%–45.0%). Biochemical examination showed a serum albumin level of 47 g/L (28–44 g/L). These findings indicated inflammation and dehydration in the animal while other indicators were within normal ranges.

Gastroscope and CT were utilized to examine the diseased monkey. Gastroscopy showed a raised lesion with a smooth surface approximately 18 cm from the incisors (above the cardia) (Figure 1). The chest spiral CT plain scan showed that the lower mediastinal oesophagus thickness was about 21.26 mm, with a corresponding narrowed lumen observed at the level of the T8–T10 vertebral body in the lower segment of the mediastinal oesophagus (Figure 2). The remainder of the thoracic cavity CT exam was normal. Combined with the results of gastroscopy and spiral CT, the diagnosis of oesophageal tumour was preliminarily confirmed. Due to the lesion obstructing the oesophagus and causing dysphagia in the rhesus monkey, we performed surgery with the written consent of the animal owner. The surgical procedure included anaesthesia (ketamine was injected intramuscularly at 10 mg/kg, after the animal was anaesthetised, inserted an endotracheal tube into the right bronchus, maintained anaesthesia with isoflurane), exposed the thoracic cavity (Figure 3A), excised the oesophageal lesion tissue and an oesophagogastric anastomosis was performed, followed by the closure of the diaphragm and thoracic cavity. The excised oesophageal tissue displayed thickening and proliferation of its inner wall, accompanied by distinct yellowish-white streaks, indicative of the presence of the pathological lesion (Figure 3B).

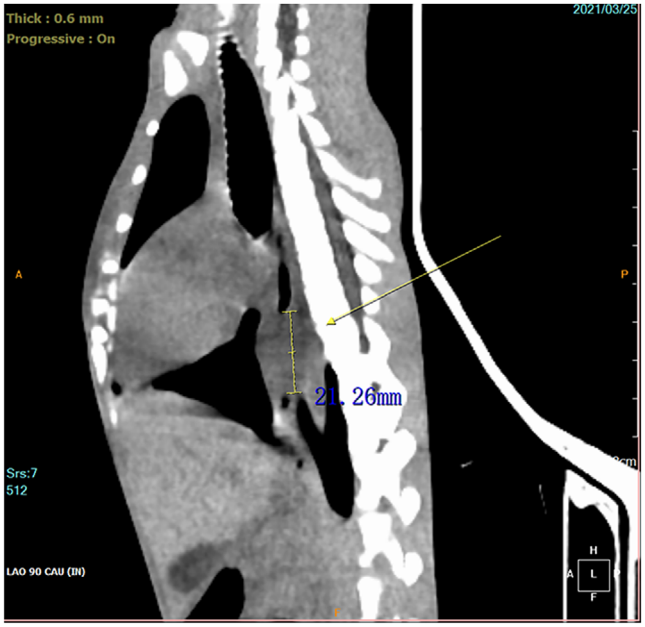

FIGURE 2 | CT scan of the rhesus monkey with ESCC, and thickness of lower mediastinal oesophagus about 21.26 mm (arrow).

Tissue samples obtained during surgery were fixed in 10% neutral buffered formalin, embedded in paraffin blocks, sectioned with a thickness of 4 μm and stained with HE. IHC was conducted using primary antibodies p63 (GB15396; Servicebio; Wuhan; China; 1:1000), cytokeratin 5/6 (CK5/6; GB14062; Servicebio; Wuhan; China; 1:200), p40 (GB150003; Servicebio; Wuhan; China; 1:1000), cytokeratin 20 (CK20; GB112050; Wuhan; China; 1:3000), cytokeratin 7 (CK7; GB12225; Servicebio; China; 1:500), cytokeratin 19 (CK19; GB11197; Servicebio; China; 1:500) and HRP labelled with the corresponding species as the secondary antibody. DAB chromogenic reagent for the histochemical kit (Servicebio, WuHan, China) was used for visualization, followed by counterstaining with haematoxylin. The expression was observed under a microscope. HE staining revealed an infiltrative tumour composed of nests of polygonal cells in the submucosa and muscularis, a few keratin pearls and necrosis (Figure 4A). These cells had frequent keratinization, large nuclear atypia and a high mitotic rate (Figure 4B). The neoplastic cells displayed multifocal strong, diffuse, cytoplasmic CK5/6, CK20 and CK7 immunoreactivity; the neoplastic cells displayed multifocal weak, diffuse, cytoplasmic CK19 immunoreactivity; the nuclei displayed multifocal strong, diffuse, cytoplasmic p63 and p40 immunoreactivity (Figure 5). The rhesus monkey in the case was positive for CK5/6, p40, p63, CK19, CK7 and CK20. Based on HE staining results and IHC findings, the rhesus monkey in this case was diagnosed as ESCC. Unfortunately, the recovery was poor due to difficulties in postoperative care and poor appetite of the animal. The owner chose to euthanize the animal on the 7th day after surgery.

3 | Discussion

SCC, also known as epidermoid carcinoma, is a neoplastic proliferation of epithelial cells that undergo squamous differentiation. SCC can occur in equids/horses (Taylor and Haldorson 2013; Elce et al. 2011), canines/dogs (De Vico et al. 1994), cattles (Al-Jameel

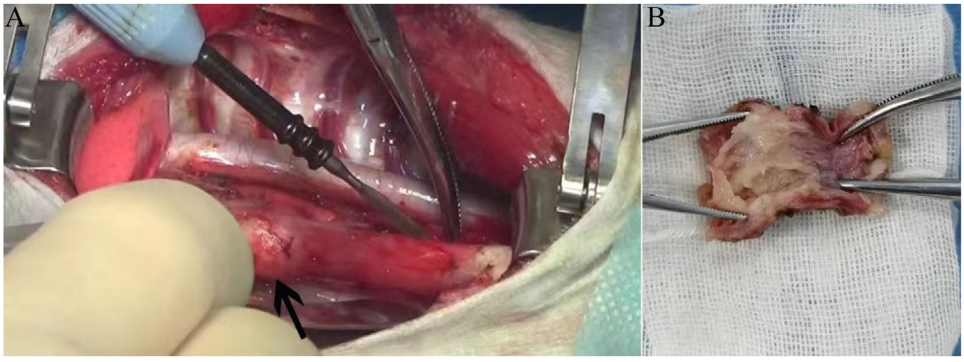

FIGURE 3 | Morphological characteristics of ESCC in the rhesus monkey. (A) The exposed thorax of a rhesus monkey. (Black arrow: location of oesophageal hyperplasia.) (B) The inner wall of the excised oesophageal tissue was thickened and proliferated with distinct yellowish-white streaks.

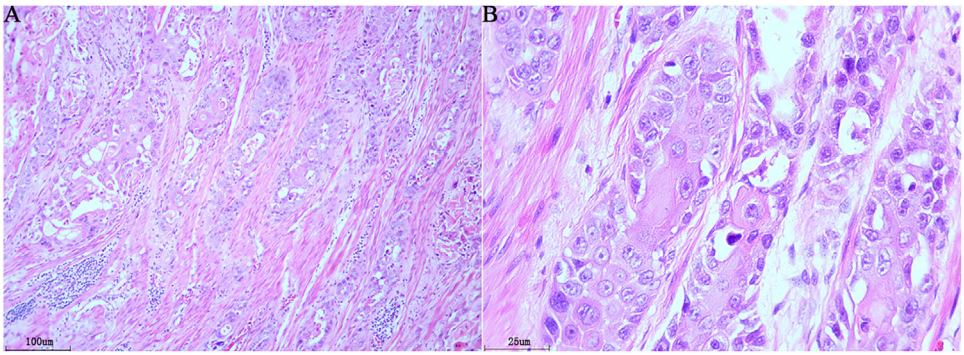

FIGURE 4 | Results of HE staining of oesophageal tumour. (A) An oesophageal tumour composed of nests of polygonal cells in the submucosa and muscularis. The scale bar, 100 μm. (B) Cells exhibited frequent keratinization and nuclear atypia. The scale bar, 25 μm.

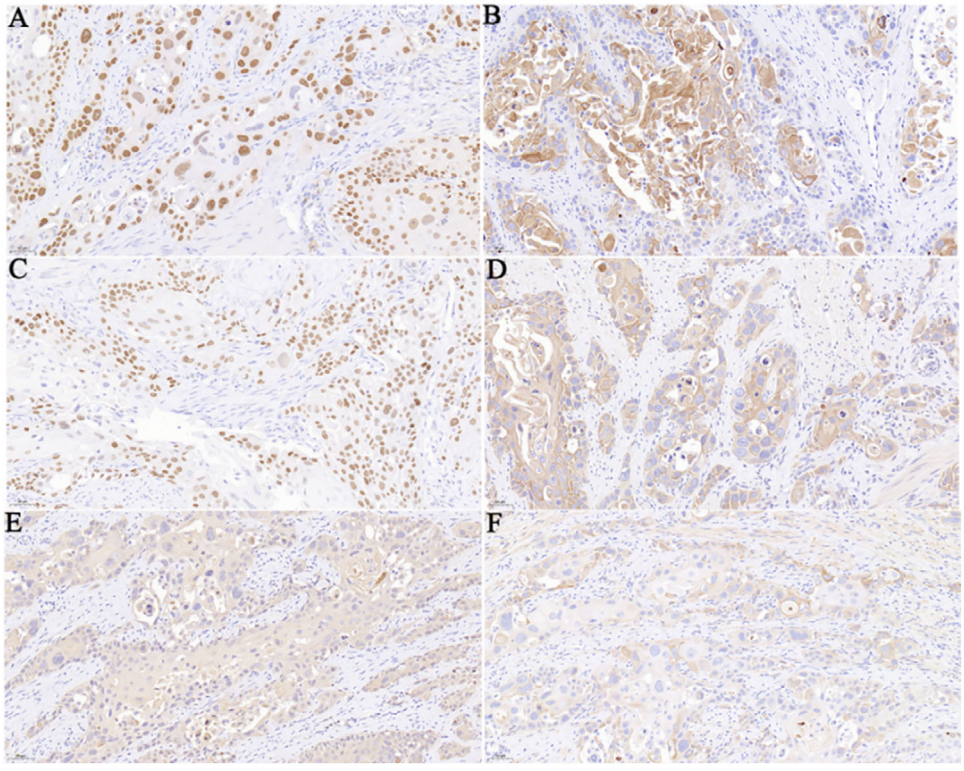

FIGURE 5 | IHC of oesophageal tumour confirming the diagnosis. (A) The nuclei displayed multifocal strong, diffuse, cytoplasmic for p63 immunoreactivity. (B) The neoplastic cells displayed multifocal strong, diffuse, cytoplasmic for CK5/6 immunoreactivity. (C) The nuclei displayed multifocal strong, diffuse, cytoplasmic p40 immunoreactivity. (D–E) The neoplastic cells displayed multifocal strong, diffuse, cytoplasmic for CK20 and CK7 immunoreactivity. (F) The neoplastic cells displayed multifocal weak, diffuse, cytoplasmic CK19 immunoreactivity. The scale bar, 50 μm.

et al. 2022) and felines/cats (Bromfield et al. 2024). ESCC is a common histological subtype of oesophageal cancer (Lam 2020). SCC has been reported in various anatomical locations including the gums, jaws, tongue, buccal pocket, oesophagus, skin, ovaries, uterus, cervix and vulva in NHPs in the literature (Morin et al. 1980; Beniashvili 1989). Predisposing factors for SCC development in animals have been reported to include sun exposure and chronic irritation (Aiello and Mays 1998). SCC of NHPs has been reported to be induced by exposure to carcinogens in the trachea, lung and oesophagus (Haddad et al. 2009). Additionally, SCC has also been associated with papillomavirus infection in NHP (Kloster et al. 1988). In this case, the result was negative by real-time polymerase chain reaction (PCR) technology (Human Papillomavirus nucleic acid typing detection kit from Jiangsu Shuoshi Biotechnology Co., Ltd.). Due to the muscular and distensible nature of the oesophagus, early-stage SCC of the oesophagus does not have typical clinical features. Symptoms caused by obstructive lesions or narrowing only become apparent when the tumour reaches a relatively advanced local stage or metastatic stage. When oesophageal lesions are suspected, a preliminary diagnosis can be established through imaging examination of the oesophagus. In humans, oesophageal cancer was most commonly found in the middle third of the oesophagus (Lam et al. 1999). Advanced ESCC often presents with mass or stenosis, whereas early neoplasms may be flat and subtle. Different stages of ESCC can be diagnosed by endoscopy (Lam 2020). Meanwhile, preoperative clinical staging was performed according to the imaging results to determine the treatment strategy.

When high suspicion of malignancy on imaging, histological assessment becomes crucial for the diagnosis and research of cancer (Lam et al. 1999). Histological types of SCC are divided into superficial and invasive categories (Gualdi et al. 2021). In this case, the tumour cells exhibited strong invasiveness and infiltrated into the muscular layer. Histological morphology of SCC can be classified as well-differentiated, moderately differentiated or poorly differentiated (Waldman and Schmults 2019). Well-differentiated SCC is characterized by squamous cells with abundant cytoplasm, intercellular desmosomal connections and a tendency to form intensely eosinophilic keratinized pearls. Conversely, poorly differentiated SCC often lacks distinct intercellular desmosomal connections, with small basophilic cells. Moderately differentiated SCC exhibits some characteristics of both well-differentiated and poorly differentiated tumours (Shanmugam et al. 2024). Pathological results, in this case, revealed an infiltrative tumour in the submucosa and muscularis composed of nests of polygonal cells. These cells have a moderate amount of amphophilic cytoplasm and oval nuclei with finely stippled chromatin; keratinization and nuclear atypia were frequently observed. These presentations are with the characteristics of moderately differentiated SCC. Proteins are involved in inflammatory and immune processes and may serve as downregulation targets for potential therapeutic modalities (Shanmugam et al. 2024). Therefore, IHC is widely used in pathological diagnosis and research, including the study of protein expression in tumours such as ESCC (Cardoso et al. 2024). Studies have demonstrated that CK5/6, p63, p40 and CK19 are considered to be markers of SCC, which are usually positively expressed in SCC. High CK5/6 expression in humans is correlated with a lower tumour grade, the positive rate of CK 5/6 in SCC was 78% (Chen et al. 2011); p63 demonstrates an 81% sensitivity to SCC (Reis et al. 2003); p40 being considered a highly specific marker for SCC with a positive rate of 96.8% in SCC cases (Tatsumori et al. 2014); and high CK19 expression has been identified as a potential indicator of malignant transformation in tissues (Yoshida et al. 2015), Chen et al. (2008) showed that CK19 was positive in 80% of SCC (Chen et al. 2008); Zhong et al. (2007) showed that CK19 positive scores in cancer tissues are significantly higher than those in corresponding distant tissues and patients with CK19 positive expression in distant tissues have a higher tumour recurrence rate and a lower survival rate compared with patients with negative CK19 expression in distant tissues (Zhong et al. 2007). CK7 and CK20 expression is associated with tumour origin and metastasis and their differential expression in tumours is also commonly used for histopathological differentiation of poorly differentiated cancers (Ramaekers et al. 1990). Expression of CK 7 was seen in the majority of cases of carcinoma, with the exception of those carcinomas arising from the colon, prostate, kidney and thymus; carcinoid tumours of the lung and gastrointestinal tract origin; and Merkel cell tumour of the skin. Ovarian metastases are usually CK7− and CK20+ (Wauters et al. 1995). Chu et al. (2000) performed an immunohistochemical study on 14 patients with oesophageal cancer and the results showed that the rate of CK7−/CK20− was 79%, CK7+/CK20− was 21%, and no CK7+/CK20+ and CK7−/CK20+ case was found (Chu et al. 2000). In this study, we aimed to explore biomarkers of ESCC in rhesus monkey by analysing the expressions of CK5/6, p63, p40, CK19, CK7 and CK20. We found that not only CK5/6, p63, p40 and CK19 were positively expressed, but CK7 and CK20 were also positively expressed in this case, which was the first discovery of ESCC in the rhesus monkey, whether these results can be applied to other monkeys needs to be studied with larger samples.

In conclusion, based on imaging and pathological comprehensive analysis, the rhesus monkey was diagnosed as ESCC in this study; however, treatment and clinical management remain a major challenge. This study provides an important reference for the diagnosis and treatment of ESCC in rhesus monkeys and also provides important information for future research on ESCC.

Author Contributions

Heling Li: methodology, writing – original draft. **Lixian Chen**: methodology, writing – original draft. **Long Zhang**: Resources. **Xing Long Chen**: resources. **Hong Wang**: project administration, supervision, writing – review and editing.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data in this study are available from the corresponding author upon reasonable request.

Transparent Peer Review

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