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Spontaneously Arising Concurrent Ileocaecal Adenocarcinoma and Renal Pelvis Transitional Cell Carcinoma in a Rhesus Macaque (*Macaca mulatta*)

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Summary

A 25-year-old, female rhesus macaque presented with a history of weight loss despite a normal appetite and supportive care. The animal was humanely destroyed due to poor prognosis. Post-mortem examination revealed a focally extensive, firm, white annular constriction at the ileocaecal junction and an incidental finding of a pale white nodule approximately 0.8 cm in diameter in the left renal pelvis. Based on the microscopical findings, ileocaecal adenocarcinoma and renal pelvis transitional cell carcinoma (TCC) was diagnosed. The use of cytokeratin (CK)-7 and -20 and uroplakin III as potential renal TCC markers was evaluated. The neoplastic cells were labelled intensely with antibodies to uroplakin III, but not to CK-7 or -20. Spontaneous intestinal adenocarcinoma has been documented in the rhesus macaque, but concurrent renal pelvis TCC is highly unusual.

Keywords

carcinoma; intestinal; renal; Rhesus macaque

The rhesus macaque (*Macaca mulatta*) is an excellent model species for the study of human ageing and disease (Roth *et al.*, 2004). Rhesus macaques have a close genetic relationship to man with a similar ageing phenotype at roughly three times the rate of man. These animals also develop spontaneously arising tumours (Bodkin *et al.*, 2003; Simmons and Mattison,

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2011). The correlation between increasing age and increasing incidence of neoplasia is well known in man. There are similar reports of an increased incidence of neoplasia in rhesus macaques >20 years of age (Uno, 1997; Uno *et al.*, 1998). Rhesus macaques are considered good comparative oncology models for investigation of spontaneous neoplasia and age-related diseases (Simmons and Mattison, 2011). One of the most commonly reported age-related diseases in rhesus macaques is intestinal adenocarcinoma (Rodriguez *et al.*, 2002). Rhesus macaques are also suggested to be an excellent candidate model for ovarian cancer chemoprevention (Brewer *et al.*, 2001). Urogenital tumours (e.g. uterine leiomyomas) are the second most commonly reported tumours in rhesus macaques and are diagnosed as fibroids in over half of women over the age of 40 (Simmons and Mattison, 2011). In man, carcinoma of the ampulla of Vater accounts for only 0.2% of all intestinal tumours, similar to less commonly diagnosed tumours in rhesus macaques (Carter *et al.*, 2008; Simmons and Mattison, 2011). In contrast, renal pelvis transitional cell carcinoma (TCC) has not been reported in this species. The present report describes the gross, microscopical and immunohistochemical features of a case of concurrent renal pelvis TCC and ileocaecal adenocarcinoma in an aged rhesus macaque.

A 25-year-old, female rhesus macaque was presented to the Yerkes National Primate Research Center (YNPRC) Veterinary Service with a history of weight loss and continuing deterioration despite a normal appetite and supportive care. The animal had been born at the YNPRC and was enrolled in several research protocols during her life. These were all approved by the Institutional Animal Care and Use Committee of Emory University. The research protocols were designed to examine the role of female hormonal state and sexual activity in male social integration, in utero transplantation of human cells into primates and to study the evolution of ageing and dementia in female primates. The animal was fed a standard diet of commercial primate chow (5037 Old World Primate Diet, PMI International, Brentwood, Missouri, USA) supplemented daily with fresh fruit and vegetables. Water was provided *ad libitum*. Room conditions included a 12:12h light:dark cycle, temperature of 24.6–29°C and 10–15 air changes per hour. On physical examination, the animal was in thin body condition (condition score of 2/5) with a pendulous abdomen, severe kyphosis and decreased extensibility of stifles and hips. Cardiac auscultation revealed a grade V/VI systolic heart murmur. Thoracic radiographs showed right-sided lung atelectasis with a pulmonary fissure line between the right cranial and caudal lung lobes. An abdominal ultrasound examination was within normal limits. No significant findings were noted on haematological and serum biochemical screening. Due to the poor prognosis and old age, the animal was humanely destroyed and a complete necropsy examination was performed. Tissue samples were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (5 µm) were stained with haematoxylin and eosin (HE).

Post-mortem examination revealed a 3.5 × 1.75 × 2.5 cm focally extensive, firm, pale white mass forming a circumferential constriction at the ileocaecal junction. The renal pelvis of the left kidney contained a single pale white nodule, approximately 0.8 cm in diameter extending into the renal medulla (Fig. 1). Other findings included multifocal fibrous adhesions between the cranial and caudal lung lobes extending to the pleura and the

diaphragm. The lung lobes frequently contained pin point black areas (pneumoconiosis) and the caudal lung lobes were multifocally atelectatic. The mitral valves had moderate endocardiosis. There was multifocal loss of articular cartilage on the right and left femoral condyles. The patella of the left knee joint was fixed by adherent fibrous connective tissue and severe kyphosis was confirmed. No other major gross findings including of the urinary bladder or ureter were noticed.

Microscopically, the nodule in the left renal pelvis was a densely cellular, unencapsulated tumour composed of neoplastic transitional epithelial cells forming papillary fronds, cords and trabeculae infiltrating the medulla and supported by fine fibrovascular stroma (Fig. 2). Neoplastic cells were polygonal with distinct cell borders and contained moderate amounts of eosinophilic granular cytoplasm. Nuclei were round to oval with finely stippled chromatin and a single, prominent, centrally-located magenta nucleolus. Anisocytosis and anisokaryosis were mild. Mitoses were 2 per 10 high power fields ($\times 400$) of view. There were many areas of squamous metaplasia within the tumour (Fig. 3). Multifocally, neoplastic cells had large cytoplasmic vacuoles with eccentric nuclei ('signet ring' cells) containing small amounts of homogeneous material or cellular debris which stained positively with periodic acid–Schiff (PAS) stain (Fig. 3). The renal interstitium was multifocally infiltrated by small numbers of lymphocytes, plasma cells, neutrophils and eosinophils. There were multifocal areas of interstitial fibrosis, mild multifocal neutrophilic glomerulitis and renal tubular degeneration and regeneration. The features of the neoplastic cells in the current case were indicative of renal pelvis TCC.

To characterize the nature of these cells further, immunohistochemistry (IHC) was performed to assess the expression of cytokeratin (CK)-7 and -20 and uroplakin III. Tissue sections were labelled with biotinylated mouse anti-human CK-7 monoclonal antibody (mAb; clone OV-TL 12/30), mouse anti-human CK-20 mAb (clone K_s20.8; both mAbs from Dako, North America Inc., Carpinteria, California, USA) and mouse anti-human uroplakin III mAb, (clone AU1; Research Diagnostics Inc., Flanders, New Jersey, USA). The labelling was 'visualized' using the streptavidin–biotin complex peroxidase method (Dako). Sections of human intestine and urinary bladder acted as positive control tissues. Negative controls were performed by omitting the primary mAbs. The neoplastic cells showed strong positive diffuse membrane labelling of surface umbrella cells and multifocal luminal labelling of uroplakin III (Fig. 4). Neoplastic cells did not express CK-7 or -20. The same IHC was performed on normal rhesus macaque kidney and urinary bladder to confirm the negative CK-7 and -20 results (data not shown).

The intestinal mass was an unencapsulated, densely cellular, infiltrative (transmural) tumour composed of epithelial cells forming acinar and tubular structures supported by thick fibrovascular stroma (desmoplastic response). Prominent mucous lakes partially lined by neoplastic epithelium, as described previously for mucinous adenocarcinoma in rhesus macaques, were present (O'Sullivan and Carlson, 2001; Rodriguez *et al.*, 2002). There was no evidence of regional lymph node metastases. The other significant microscopical lesions in this case were multifocal amyloid deposition in the spleen and liver, confirmed by positive Congo red staining.

Neoplastic disease in captive rhesus macaques is becoming more prevalent as a result of emphasis on ageing research. There are few comprehensive retrospective studies of spontaneously arising neoplasia in rhesus macaques (Jones and Casey, 1981; Rodriguez *et al.*, 2002; Simmons and Mattison, 2011). A recent survey of the incidence of spontaneously arising neoplasia in captive rhesus macaques over a 28-year period at the Wisconsin National Primate Research Center showed that the gastrointestinal system was most commonly affected, accounting for 48.8% of all tumours identified, and intestinal adenocarcinomas (37%) were the most prevalent among these. Intestinal adenocarcinoma most commonly affected the colon (41.7%) followed by the ileocaecal junction (30%). Renal cell carcinoma accounted for 6.55% of the urogenital tumors (23.5%) identified in that survey (Simmons and Mattison, 2011). The average age of macaques with intestinal adenocarcinoma was 22.2 years. The signalment, clinical and gross findings in the present report, including old age, a history of weight loss and circumferential constriction of the intestinal wall were consistent with earlier reports of intestinal adenocarcinoma in rhesus macaques (O'Sullivan and Carlson, 2001; Rodriguez *et al.*, 2002). Colonic carcinomas are the second most common cause of cancer mortality in man and these tumours develop from adenomatous polyps that form polypoid or fungoid masses in the caecum or ascending colon and circumferential constriction of the distal colon (Stricker and Kumar, 2010).

Primary renal tumors are reported rarely in rhesus macaques. One retrospective study of 17 non-human primates reported 10 cases of renal carcinoma, four of renal adenoma, one nephroblastoma, one hamartoma and one case of transitional cell papillomatous hyperplasia (Jones and Casey, 1981). These tumors were 0.1–10 cm in diameter. The most frequent clinical signs were lethargy, weight loss, depression and dehydration. Spontaneously arising renal cell carcinoma (Chapman and Allen, 1968), urinary bladder TCC (Chesney and Allen, 1973) and renal cortical adenoma (Kaur *et al.*, 1968) have been documented in rhesus macaques. There is a single case report in which four concurrent tumours, namely osteogenic sarcoma, renal cell carcinoma, renal pelvis TCC and basal cell carcinoma were diagnosed in an irradiated rhesus macaque (Valerio *et al.*, 1968). The authors found a 0.2 cm nodule in the right renal pelvis that was diagnosed as renal pelvis TCC. In the present case, the left renal pelvis nodule was 0.8 cm in diameter and was discovered as an incidental finding. Renal pelvic epithelial hyperplasia was considered as the primary differential during necropsy examination. There are few reports in which urinary bladder TCC was induced by irradiation (Allen *et al.*, 1970) or feeding 2- naphthylamine (Conzelman *et al.*, 1969) in rhesus macaques. However, spontaneously arising renal pelvis TCC has not been reported for this species. Renal pelvis TCC has been reported in dogs (Militerno *et al.*, 2003), cats (Hanzlicek *et al.*, 2012) and cattle (Vitovec, 1977). In dogs, proteinuria and haematuria or normal urinalysis can be present (Militerno *et al.*, 2003). In man, tumours of the renal pelvis account for 7–8% of all renal malignancies and >90% are of transitional cell origin. Risk factors for TCC of the upper urinary tract include environmental and occupational hazards, chemotherapeutic exposure and previous history of urinary bladder or ureteral carcinomas (Gupta *et al.*, 2008). The rhesus macaque in this report was housed in an indoor research facility, fed a controlled diet and was not exposed to environmental carcinogens.

The molecular and genetic basis of upper urinary tract urothelial carcinoma appears similar to that of urothelial carcinomas of the urinary bladder, and involves tumour suppressor genes such as *p53* and several loci on chromosome 9 in 50–75% of human patients (Gupta *et al.*, 2008). Microsatellite instability and loss of mismatch repair proteins MSH2, MLH1 and MSH6 can be seen in 20–30% of cases (Blaszyk *et al.*, 2002). Frequent replication errors at microsatellite loci have been described in patients with multiple primary malignant lesions (Hori *et al.*, 1994). The morphology of urothelial carcinomas, particularly poorly differentiated tumours or tumours in metastatic sites, usually overlaps significantly with poorly differentiated non-urothelial carcinomas.

In people, expression patterns of CK-7 and -20 are often used to distinguish high grade urothelial carcinoma from renal cell carcinoma; the majority of renal cell carcinomas do not express these markers. However, urothelial carcinomas express CK-7, but CK-20 expression is variable with reports ranging from 22–50% positivity (Chu *et al.*, 2000; Mhawech *et al.*, 2002; Skinnider and Amin, 2005). Uroplakin III, a transmembrane protein expressed by urothelial lining cells, is another specific and moderately sensitive marker of urothelial lesions (Parker *et al.*, 2003). Currently, no markers are used widely to confirm the urothelial origin of tumours in rhesus macaques. Therefore, the potential use of CK-7, -20 and uroplakin III was evaluated in the current case. The renal pelvis TCC was labelled intensely for uroplakin III, but not CK-7 or -20. The negative CK labelling may indicate a difference to man or lack of antibody cross-reactivity with macaque CKs. The data presented here enhance our knowledge of the gross, microscopical and immunohistochemical features of renal pelvis TCC in the rhesus macaque.

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Fig. 1.
An 0.8 cm diameter, pale white nodule in the renal pelvis of the left kidney extending into the medulla.

Fig. 2.

The renal pelvis nodule is composed of neoplastic transitional epithelial cells forming papillary fronds, cords and trabeculae infiltrating the medulla. HE. $\times 5$.

Fig. 3.

The renal pelvis tumour includes areas of squamous metaplasia (arrowhead) and cells with large cytoplasmic vacuoles with eccentric nuclei ('signet ring' cells) containing small amounts of homogeneous material or cellular debris. HE. $\times 200$. Inset: large cytoplasmic vacuoles (arrow) containing small amounts of PAS-positive material. PAS. $\times 600$.

Fig. 4.
Intensive immunolabelling of the surface membrane (*) and a luminal (arrowhead) population of neoplastic cells with uroplakin III. IHC. $\times 200$.