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SHORT PAPER

Immunohistochemical Characterization of a Squamous Cell Carcinoma in a Harbour Porpoise (*Phocoena phocoena*) from German Waters

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Summary

Neoplastic diseases in cetaceans are considered relatively uncommon. This report describes a gastric squamous cell carcinoma in an adult male harbour porpoise (*Phocoena phocoena*) stranded on the North Sea coast of Schleswig–Holstein, Germany. The tumour arose from the squamous epithelium of the first compartment of the stomach and metastases were found in the pulmonary and retropharyngeal lymph nodes, liver, lung and brain. Neoplastic epithelial cells expressed cytokeratin (CK) 5, CK6 and CK10. This pattern of CK expression did not differ from that of normal porpoise squamous gastric mucosa and partially shares the CK profile of human oesophageal epithelium. Tumour cells strongly expressed p53, suggesting a possible role for this tumour suppressor gene in tumourigenesis.

Keywords: cytokeratin; harbour porpoise; immunohistochemistry; *Phocoena phocoena*; squamous cell carcinoma

Over the past two decades there has been increasing impact of human activity on the marine ecosystem and public awareness of this has resulted in increased management of this environment (ASCOBANS, 2000; Gilles *et al*., 2009). To that end, systematic pathological investigations have been performed on harbour porpoises (*Phocoena phocoena*) from different European waters (Baker and Martin, 1992; Siebert *et al*., 2001; Jauniaux *et al*., 2002; Jepson *et al*., 2005; Siebert *et al*., 2006). The effect of pollutants on the health status of porpoises has been associated with an increased occurrence of infectious diseases as well as a reduced function of the immune and endocrine systems in these animals (Siebert *et al*., 1999; Beineke *et al*., 2005; Jepson *et al*., 2005; Das *et al*., 2006). In contrast, relatively few cases of neoplastic disease have been reported in harbour porpoises or other marine mammals. These cases include a cutaneous squamous papilloma in a harbour porpoise, narwhale and killer whale (Geraci *et al*., 1987), one gastric adenocarcinoma with multiple metastases in a harbour porpoise (Breuer *et al*., 1989), one adenocarcinoma of undetermined origin (Baker and Martin, 1992) as well as an adrenal cortical adenoma in two Atlantic white-sided dolphins (Newman and Smith, 2006). Types and distribution of tumours in marine mammals are assumed to be similar to those of domestic species (Landy, 1980; Howard *et al*., 1983; Geraci *et al*., 1987; Newman and Smith, 2006). The present case report describes a metastatic squamous cell carcinoma originating from the non-glandular part of the stomach in a harbour porpoise.

An adult male harbour porpoise, 5–6 years of age, measuring 144 cm in length and weighing 42.0 kg was stranded on the western shore of the North Sea island of Pellworm, Germany. The carcass was stored at −20 °C until necropsy examination was performed according to the standardized European protocol (Siebert *et al*., 2001). The carcass was weighed and measured, and four teeth from the middle of the lower jaw were removed for determination of age by

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counting the annual growth layers (Lockyer, 1995). The nutritional state was judged based on the weight of the total blubber and muscle (along the dorsal column) as well as blubber thickness and the degree of muscle atrophy. The blubber thickness was measured in four different locations (sternal, caudodorsal, caudolateral and caudoventral to the dorsal fin; Siebert *et al.*, 2001).

Samples were collected from different organs and tissues, fixed in 10% neutral buffered formalin, processed by routine methods, embedded in paraffin wax, sectioned (5 μm) and stained with haematoxylin and eosin (HE). Immunohistochemistry (IHC) was performed with a panel of murine monoclonal antibodies (all from DakoCytomation, Hamburg, Germany) specific for cytokeratin (CK): clone AE1/AE3 specific for CK1–CK8, CK10, CK13–CK16 and CK19, diluted 1 in 500 in phosphate-buffered saline (PBS, pH 7.1); clone MNF 116 specific for CK5, CK6 and CK18, diluted 1 in 100 in PBS; clone 34βE12 specific for CK1, CK5, CK10 and CK14, diluted 1 in 500 in PBS; clone D5/16B4 specific for CK5 and CK6; clone OV-TL12/30 specific for CK7; clone DE-K10 specific for CK10; clone Ks20.8 specific for CK20, all diluted 1 in 100 in PBS. Sections were also tested with clone DO-7 specific for the tumour suppressor protein p53 (diluted 1 in 50 in PBS) and a rabbit polyclonal antibody specific for factor VIIIRa (DakoCytomation), diluted 1 in 200 in PBS. IHC was performed as described previously (Döpke *et al.*, 2007).

Due to lack of knowledge of the cross-reactivity of these antibodies and distribution of CK expression in this species, sections of normal gastric mucosa of a harbour porpoise were included in the experiment. Sections of canine mammary tissue (for clone 34βE12), canine prostate gland (for OV-TL12/30), canine small intestine (for clone Ks20.8) and canine skin (for the remaining antibodies) were used as positive controls. A feline cutaneous squamous cell carcinoma was used as control for p53 expression. For negative control purposes the primary antibody was replaced by ascites fluid from non-immunized BALB/cJ mice (Biologo, Kronshagen, Germany) or normal rabbit serum (Sigma Chemie, Taufkirchen, Germany).

Parasites identified during the necropsy examination were fixed in 70% ethanol prior to identification (Lehnert *et al.*, 2005). In addition, samples of lung, liver, kidney, spleen, intestine, intestinal lymph nodes and grossly visible lesions were submitted for bacteriological examination (Siebert *et al.*, 2009). Serological tests for morbillivirus antibodies were performed according to Müller *et al.* (2000).

The porpoise was emaciated with muscular atrophy (weight of the Longissimus dorsi muscle 2.18 kg) and low blubber thickness (12–18 mm, measured at nine different locations; blubber weight of 8.4 kg). An ulcerated, firm nodular mass (1.5 cm in diameter) was present in the first stomach compartment (pars proventricularis). On cut surface grey–white poorly demarcated and firm tissue was found to infiltrate the stomach wall. A moderate parasitic nematode load was present in the first compartment of the stomach and this was associated with two foci of mucosal ulceration measuring 3×2.5 and 4×5 cm^2, respectively. There were numerous partly elevated and firm nodules (up to 4 cm diameter) with a white homogenous cut surface within the lung. Brain and liver were grossly normal. Pulmonary and retropharyngeal lymph nodes were markedly enlarged with a bulging, homogeneous white cut surface.

Microscopical examination revealed a poorly differentiated squamous carcinoma originating from the non-glandular part of the stomach (Fig. 1A) and infiltrating the gastric wall (Fig. 1B). The tumour cells formed nests and cords of non-keratinizing oval to polygonal epithelial cells. Tumour cell emboli were detected in lymphatic vessels. The nuclei were round to oval with marked anisokaryosis and occasional karyomegaly (Fig. 2). Mitoses ranged from 0 to 2 per high power (×40 objective) field. Metastatic tumour cells displayed almost no squamous differentiation and were present in the lung, liver, cerebral cortex and the pulmonary and retropharyngeal lymph nodes. Areas of necrosis were frequently present within these metastatic nodules.

Immunohistochemical examination showed the neoplastic cells to have strong labelling with antibody clones AE1/AE3 (CK1–CK8, CK10, CK13–CK16 and CK19), MNF 116 (CK5, CK6, CK8, CK17 and CK19), 34βE12 (CK1, CK5, CK10 and CK14) and D5/16B4 (CK5 and CK6) (Fig. 3A). The same pattern of expression was displayed by the squamous epithelium of the normal gastric mucosa. Normal gastric mucosa and single small clusters of tumour cells were labelled with the clone DE-K10 specific for CK10 (Fig. 3B). There was no expression of CK7 or CK20 by normal or neoplastic cells. Factor VIIIRa expression was restricted to vascular endothelial cells. Almost all tumour cells displayed intense nuclear labelling for p53 (Fig. 4).

Additional microscopical findings included severe pulmonary oedema, moderate diffuse lymphoplasmacytic inflammation of the pancreatic duct and mild to moderate diffuse lymphoplasmacytic cholecystitis. Parasitological investigations revealed moderate numbers of *Anisakis simplex* in the first compartment of the stomach; low numbers of *Pseudalius inflexus* in the right atrium, the oesophagus and lung; moderate numbers of *Torynurus convolutus* in the oesophagus and lung; low