

NOTE

Multicentric plasmacytoma in a harbor porpoise *Phocoena phocoena* off the coast of Whidbey Island, Washington State, USA

Barry H. Rickman^{1,2,3,*}, Tim Morgan⁴, Matthew Klope², Susan Berta², Sandra Dubpernell², Howard Garrett², Mary Jo Adams², Stephanie A. Norman^{2,5}

¹Faculty of Veterinary Science, University of Sydney, Camden, New South Wales 2570, Australia

²Central Puget Sound Marine Mammal Stranding Network, Whidbey Island, Washington 98249, USA

³Sound VetPath, Edmonds, Washington 98020, USA

⁴Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, Mississippi 39762, USA

⁵Marine-Med: Marine Research, Epidemiology, and Veterinary Medicine, Bothell, Washington 98021, USA

ABSTRACT: Necropsy of a female adult pregnant harbor porpoise *Phocoena phocoena* revealed a multicentric plasmacytoma. The plasmacytoma infiltrated the cranial lung lobes, mediastinal lymph nodes and the spleen. Diagnosis was based on gross, histopathologic and immunohistochemical studies. Histopathology revealed a diffuse proliferation of atypical pleomorphic neoplastic round cells with plasmacytic features. Positive immunohistochemistry with anti-CD79a and anti-CD20 antibody markers and anti-multiple myeloma oncogene 1 (MUM-1) for plasmacytoma confirmed this neoplasm to be of B-cell origin. This is the first recorded case of a plasmacytoma in a harbor porpoise. Routine viral screening was negative via standard PCR for herpesvirus and reverse transcriptase PCR for morbillivirus. Retroviral screening was not performed.

KEY WORDS: Cancer · Neoplasia · Cetacean · Harbor porpoise · Multicentric anaplastic plasmacytoma · *Phocoena phocoena* · Marine mammal

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INTRODUCTION

Cetaceans are a group of marine mammals consisting of dolphins, whales and porpoises. The incidence of reported neoplasia is uncommon in this group of animals (Newman & Smith 2006). Few lymphoid and hematopoietic neoplasms have been described in dolphins and whales, with no recorded reports in porpoises (Gulland et al. 2001, Newman & Smith 2006). There is growing concern regarding the link between neoplasia and environmental carcinogens and pollutants, especially in ocean ecosystems (Hansen et al. 2004, Fair et al. 2010, Bossart 2011); there-

fore, it is important to have a surveillance program that documents trends of neoplasia in marine mammals. This report is the first morphologic description and immunophenotyping of a lymphoid neoplasm, specifically a plasmacytoma in a harbor porpoise *Phocoena phocoena*.

METHODS AND RESULTS

A fresh dead harbor porpoise with no overt signs of injury was reported at Maxwelton Beach, South Whidbey Island on 1 November 2013. A post-mortem

*Corresponding author: barry.rickman@gmail.com

examination was performed by members of the Central Puget Sound Marine Mammal Stranding Network on 3 November 2013.

On gross examination, the adult female porpoise was in poor nutritional condition, weighing 68.6 kg. The porpoise was determined to be in good post-mortem condition based on fresh odor and lack of tissue discoloration or bloating (Geraci & Lounsbury 2005). There were several firm, raised nodules in the blubber and muscle of the right abdominal wall containing encysted parasites. Within the left uterine horn was an approximately 1-mo-old early gestational fetus measuring 5.5 cm in length by 1.8 cm in width (Börjesson & Read 2003). The dorsal lungs contained mottled areas of cream colored spots. The ventral tips of both lung lobes contained multiple tan nodules measuring up to 3.5 cm in diameter. Multifocal mediastinal lymph nodes were enlarged. The prescapular, mediastinal and colonic lymph nodes were markedly enlarged and the spleen contained 2 poorly defined intraparenchymal nodules. The first chamber of the stomach contained nematode worms.

The following tissues were collected and saved in 10% neutral buffered formalin for fixation: submandibular lymph node, prescapular lymph node, mesenteric lymph node, mediastinal lymph node, colonic lymph node, skeletal muscle, eye, urinary bladder, diaphragm, trachea, heart, lung, liver, spleen, kidney, stomach, intestine, uterus, adrenals, brain, spinal cord and tonsils. The bone marrow was not collected. These samples were embedded in paraffin, sectioned at 4 μm , and stained with hematoxylin and eosin.

Histologically, the affected pulmonary interstitium (Fig. 1A), splenic nodules and the mediastinal lymph nodes were effaced by neoplastic cells (Fig. 1B). All neoplastic cells in the 3 organs have similar cellular features. In the case of the lymph node, the entire mediastinal lymph node cortical, paracortical and medullary architecture are diffusely effaced by an infiltrative, densely cellular neoplasm composed of sheets of pleomorphic round cells (Fig. 1B). Neoplastic cells had distinct borders and moderate amounts of hypereosinophilic cytoplasm, and nuclei were either centrally located or eccentric, round with coarsely stippled chromatin, occasionally hyperchromatic, and occasionally had 1 or 2 prominent nucleoli. There were occasional perinuclear halos. Nuclei were on average 6.4 μm in diameter, approximately 1.3–1.4 times the diameter of an erythrocyte (Valli 2007). Occasional binucleate and multinucleate cells were present. There was marked anisocytosis and anisokaryosis (Fig. 1B). Mitoses averaged 6 per high power field (400 \times). Multifocally, neoplastic cells were

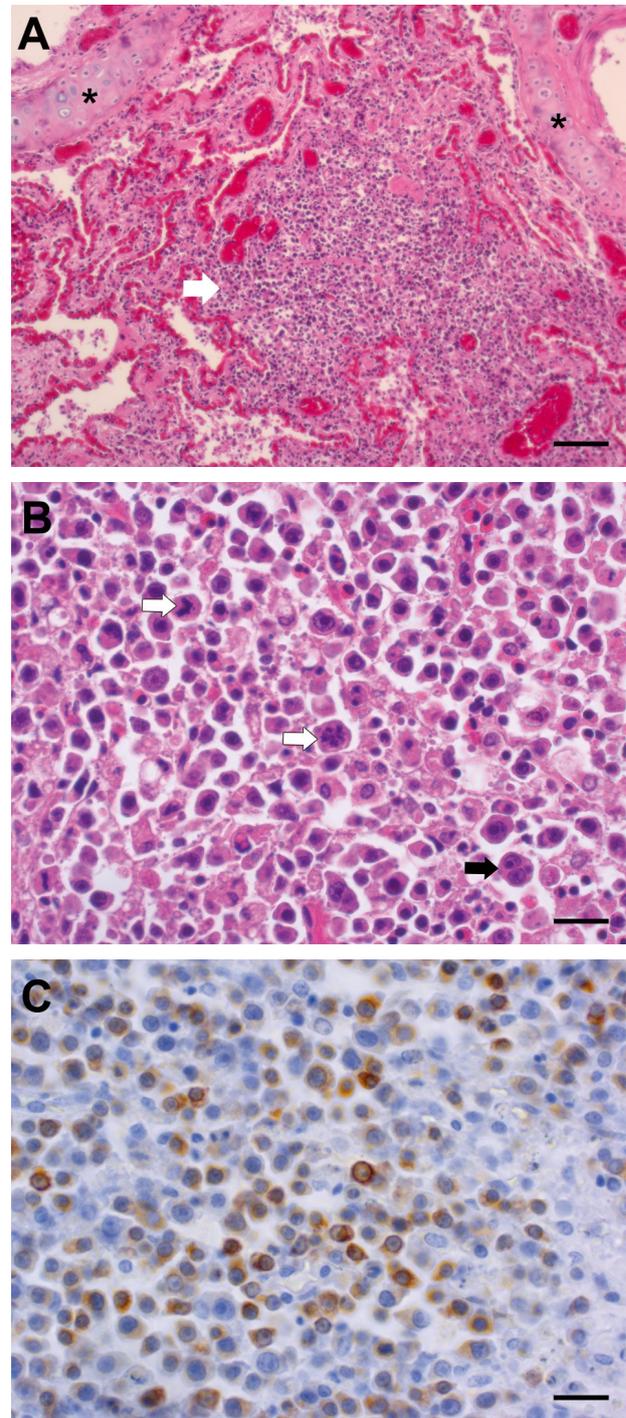


Fig. 1 (A) Lung hematoxylin and eosin. The pulmonary interstitium is infiltrated and effaced by sheets of pleomorphic neoplastic plasma cells (white arrow). Asterisk indicates bronchi cartilage. Scale bar = 100 μm . (B) Mediastinal lymph node (cortex) hematoxylin and eosin. The lymph node architecture is effaced by sheets of neoplastic plasma cells. There is marked anisocytosis and anisokaryosis. The black arrow indicates a binucleate cell and the white arrows indicate a bizarre mitotic. Scale bar = 20 μm . (C) Anti-CD79a immunohistochemistry staining. Neoplastic plasma cells have variable moderate to strong cytoplasmic staining. Scale bar = 20 μm

within vessels and lymphatics. Degeneration and necrosis of neoplastic cells were present. In some lymph nodes, there were multifocal areas of capsular invasion by neoplastic cells and there were extracapsular aggregates of neoplastic cells. Similar neoplastic cells in the lungs disrupted and infiltrated the pulmonary parenchyma, and in the spleen, infiltrated, expanded and disrupted the normal splenic parenchyma.

The colonic wall was infiltrated by predominantly neutrophils and there was no evidence of neoplastic cells in the colon. There were hepatic granulomas with intralesional trematode eggs along with a marked chronic mixed cell capsulitis in the liver. Neoplastic cells were not observed within the other organ systems examined nor the fetus. Immunohistochemistry with T-cell markers, CD3, B-cell markers CD79a and CD20, and multiple myeloma oncogene 1 (MUM-1) for plasmacytoma was performed to characterize the cell origin. For immunohistochemical staining, formalin-fixed, paraffin-embedded tissues were cut into 4 µm (CD20, MUM-1) or 5 µm (CD3, CD79a) sections and placed on charged glass slides. Slides were deparaffinized in xylene and ethanol baths and rinsed with de-ionized water. Antibodies and an outline of immunohistochemical procedures are included in Table 1. Sections were counterstained with hematoxylin (Sigma) and coverslipped.

Approximately 80% of the neoplastic cell population showed moderate, diffuse, cytoplasmic immunopositivity for CD79a in the mediastinal lymph node sections. Approximately 80% of the neoplastic cell population showed moderate, membrane staining for CD20, and 70% of the neoplastic cell population showed strong nuclear staining with a lighter diffuse cytoplasmic staining for MUM-1. Approximately 20% of normal-appearing lymphocytes were immunopositive for CD3, indicating these were a combination of resident lymph node T-cells and/or infiltrating T-cells. There was no staining with the negative

immunoglobulin G control in the lymph node. The moderate specific staining with all the antibodies suggests that there is cross-reactivity of the mouse-produced CD79a and rabbit-produced CD3, CD20 and MUM-1 antibodies with formalin-fixed harbor porpoise tissue. This supports the suitability of immunolabeling with antibodies from another species of origin as previously described with dolphins. There are limited reports of polyclonal lymphoid antibodies that have been used with cetaceans, particularly in dolphins, and not at all in porpoises (Bossart et al. 1997, Jaber et al. 2003).

Viruses that cause cancer, oncogenic viruses, have been associated with a variety of animal species (Zachary 2017) and may be associated with the development of hematopoietic neoplasms, especially lymphomas (Bossart et al. 1997, Saha & Robertson 2011, Efirid et al. 2014). However, few infectious agents have been associated with cancer in marine mammals, whereas in domestic and wild animals, retroviruses have been associated with lymphoma or leukemia in mice (Rosenberg & Jolicœur 1997), cats (Hartmann 2012), koalas (Denner & Young 2013) and gibbon apes (Gallo et al. 1978). In California sea lions *Zalophus californianus* and humans, herpesviruses have been associated with the presence of B-cell lymphomas (Klein 1974, Venn-Watson et al. 2012). Morbilliviruses are known to induce immune suppression (de Vries et al. 2015), leaving the host susceptible to secondary infection and severe illness and are commonly documented in marine mammals in large outbreaks (Rowles et al. 2011). Therefore, molecular detection by PCR screening was performed for cetacean morbillivirus (reverse transcriptase PCR; Tong et al. 2008) and herpesvirus (standard PCR; VanDevanter et al. 1996) on tumor sections of the mediastinal lymph nodes. Sections were submitted to the Zoological Medicine and Wildlife Disease Laboratory at the University of Florida, College of Veterinary Medicine (Gainesville, FL, USA) for

Table 1. Antibodies and immunohistochemical methods. HIER: heat-induced epitope retrieval; LSAB: labeled streptavidin biotin; MUM-1: multiple myeloma oncogene 1; HRP: horseradish peroxidase

Antibody (clone)	Source	Host species	Dilution	Incubation time (min)	Antigen retrieval	Detection system
CD79a (HM57)	ABCAM AB62650	Mouse monoclonal	1:100	60	Steamer, pH 6, 30 min	LSAB2 (DAKO-K0675)
CD3 (polyclonal)	DAKO-AO452	Rabbit polyclonal	1:100	60	Steamer, pH 6, 30 min	LSAB2 (DAKO-K0675)
CD20 (polyclonal)	Neomarker RB-9013-P1	Rabbit polyclonal	1:300	30	HIER Steamer, 30 min	BioCare Medical Polymer-HRP (rabbit RC542H)
MUM-1 (BC5)	Biocare (CRM352)	Rabbit monoclonal	1:400	30	HIER Steamer, 30 min	BioCare Medical Polymer-HRP (rabbit RC542H)

testing and all were negative (Norman et al. 2017). Retrovirus screening was not performed.

Based on the histological findings in combination with the positive immunohistochemistry with CD79a, CD20 and MUM-1, we concluded that this is a B-cell lineage neoplasm most consistent with multicentric plasmacytoma, with closest features to the well-described canine polymorphous-blastic type plasmacytoma (Platz et al. 1999, Zachary 2017). Eight percent of canine plasmacytomas were reported as positive for all 3 markers, CD79a, CD20 and MUM-1 (Ramos-Vara et al. 2007).

DISCUSSION

Plasmacytic neoplasms are uncommon neoplasms that have been described in many animal species and in humans (Zachary 2017). Three types of plasma cell neoplasms were considered to further characterize this neoplasm: extramedullary plasmacytoma, multiple myeloma and lymphoplasmacytic lymphoma. Extramedullary plasmacytomas are monoclonal proliferations of terminally differentiated B-cells that do not involve the bone marrow. Multiple myelomas are plasmacytomas that arise in the bone marrow and can have extramedullary sites. A diagnosis of multiple myeloma requires finding at least 2 of the following abnormalities: marked increased numbers of plasma cells in the bone marrow, monoclonal gammopathy, evidence of osteolysis and light-chain proteinuria. We did not collect bone marrow samples therefore we cannot completely rule out a diagnosis of multiple myeloma. Lymphoplasmacytic lymphoma is classified as a small-cell, low-grade lymphoma with some cells having plasmacytoid differentiation that typically involves the bone marrow (Naderi & Yang 2013). Our case consists of a more aggressive higher-grade neoplasm with a lack of small lymphocytes, therefore ruling out the possibility of lymphoplasmacytic lymphoma. Based on histological features, this neoplasm most closely resembles a polymorphous-blastic type plasmacytoma, as described in the dog (Platz et al. 1999). The main features described with this subtype are anisocytosis, large numbers of giant cells and lack of a perinuclear halo. We do observe marked anisocytosis but we do not have large numbers of giant cells and a perinuclear halo is present in our case. Unlike the dog, this is the first report of this tumor in a porpoise and its features do not exactly fit into the dog-type classification.

Immunohistochemistry for multiple myeloma oncogene 1/interferon regulatory factor 4 (MUM1/IRF-4)

can be useful in detection of multiple myeloma and plasmacytomas. MUM1/IRF4 is an interferon regulatory factor that is involved in lymphoid cell differentiation via its role in light-chain rearrangement at the pre-B stage of lymphocyte maturation (Adelman et al. 2014). The MUM1/IRF4 antibody has been used successfully in dogs and cats in the diagnosis of plasmacytoma (Ramos-Vara et al. 2007, Sykes et al. 2017), but has not been reported with marine mammals. The MUM-1 nuclear staining pattern in this porpoise is similar to that described in the literature with dogs (Ramos-Vara et al. 2007). This is the first report of a MUM-1 antibody successfully staining a B-cell lineage neoplasm in a cetacean.

Plasmacytomas have not been reported in cetaceans (Gulland et al. 2001, Newman & Smith 2006). There are few case reports of haematopoietic neoplasms in cetaceans with lymphoma being most commonly described (Gulland et al. 2001, Newman & Smith 2006). Cetacean lymphomas reported include lymphoma in beluga whales *Delphinapterus leucas* (Martineau et al. 2002a), Hodgkin's lymphoma in a fin whale *Balaenoptera physalus* (Martineau et al. 2002a, Newman & Smith 2006) and in a killer whale *Orcinus orca* (Yonezawa et al. 1989), immunoblastic lymphoma in Atlantic bottlenose dolphin *Tursiops truncatus*, an Atlantic spotted dolphin *Stenella frontalis*, a pantropical spotted dolphin *Stenella attenuata* (Bossart et al. 1997) and a Pacific white-sided dolphin *Lagenorhynchus obliquidens* (Howard 1983), primary uterine T-cell lymphoma in an Atlantic spotted dolphin *Stenella frontalis* (Díaz-Delgado et al. 2015) and central nervous system T-cell lymphoma in a common dolphin *Delphinus delphis* (Arbelo et al. 2014). In addition, haematopoietic neoplasms with possible links to environmental pollutants have been reported in cetaceans. A hepatosplenic lymphoma in a bottlenose dolphin had high levels of polychlorinated biphenyl (PCB) congeners (Jaber et al. 2005) and a thymic lymphoma in a beluga whale had high concentrations of PCBs and polaromatic hydrocarbons (Martineau et al. 2002b).

There is a growing concern for the increased trends of neoplasia in marine mammals and the possible link with environmental pollutants. Marine mammals are exposed to a variety of persistent organohalogen compounds that bioaccumulate in marine ecosystems and this can result in high tissue contaminant concentrations over their lifetime (Aguilar et al. 2002, Hansen et al. 2004, Fair et al. 2010). The levels of organic pollutants in this porpoise were investigated, with the possible association of exposure to carcinogenic toxins of this harbor porpoise described in another publication (Norman et al. 2017).

CONCLUSION

This is the first report of a B-cell lineage neoplasm with features most consistent with a plasmacytoma described in the harbor porpoise, as well as in any cetacean. Plasmacytoma should be considered as a differential diagnosis with neoplastic diseases in the harbor porpoise.

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