Comparative Medicine
Copyright 2014
by the American Association for Laboratory Animal Science

Vol 64, No 6
December 2014
Pages 421–423

Case Report

Pancreatic Carcinoma in an African Clawed Frog (*Xenopus laevis*)

Adam W Stern,[1,*] Sarah O Allison,[2] and Caroline Chu[3]

This report describes the histologic features of a pancreatic carcinoma in an adult female African clawed frog (*Xenopus laevis*). The animal was found to be in poor body condition and subsequently euthanized for a complete necropsy. Histologically, the pancreas was effaced by packets of polyhedral cells consistent with a pancreatic islet cell carcinoma. Metastatic disease was not identified. Pancreatic tumors are uncommon in amphibians, and this report is the first to describe a pancreatic carcinoma in an African clawed frog.

The African clawed frog (*Xenopus laevis*) is one of the world's most commonly used frog species in developmental biology, cell biology, toxicology, and neuroscience. Neoplastic diseases in *Xenopus* have rarely been reported in the literature but include hepatomas, teratomas, renal carcinoma, fibroma, fibrosarcoma, nephroblastoma, ovarian dysgerminoma, melanophoromas and lymphoma.[1,2,5,6,10,14] Although hyperplasia of pancreatic islets in *Xenopus* has been described, pancreatic neoplasms in these frogs have not previously been reported.[15] Pancreatic carcinomas have been reported to occur in hybrid species of pond frogs of the *Pelophylax* (previously *Rana*) genus.[9] This report describes the first documented occurrence of a pancreatic carcinoma in *X. laevis*.

Case Report

An adult, sexually mature female *X. laevis* was found to be in poor body condition during the daily animal health check. This animal was group housed in a 189-L tank with 13 other adult female frogs in accordance to the *Guide for the Care and Use of Laboratory Animals*.[8] This frog had been purchased from a commercial vendor (*Xenopus* Express, Brooksville, FL). The frogs were housed within a custom-designed flow-through housing system (Aquatic Habitats, Apopka, FL) with a reverse-osmosis water purification system (Millipore RIOS, Billerica, MA). After the water underwent purification, 37.5 g/L sea salt (Oceanic Sea Salt, Central Aquatics, Franklin, WI) was added to the conductivity tank, and 16 g/L sodium bicarbonate (Sodium Bicarbonate, Feed Products and Service Company, St Louis, MO) was added to the pH tank. Water quality parameters within the housing system were maintained as follows: water temperature, 17 to 18 °C; pH range, 7.7 to 7.9; and conductivity, of 745 to 1013 µS. Frogs were fed commercial brittle (Nasco, Ft Atkinson, WI) 3 times each week. This colony of frogs was used for egg and oocyte collection. Two months prior to clinical presentation, the index frog had been stimulated with human chorionic gonadotropin to induce egg laying. The University of Illinois animal care program is accredited by AAALAC. All animal experiments were approved by the University of Illinois at Urbana-Champaign IACUC.

On examination, the frog was alert but emaciated, with bony prominence of the spine and pelvis. There were no other lesions or signs of trauma noted. Due to its poor body condition, the frog was euthanized by immersion within a buffered 5 g/L tricaine methane sulfonate solution (Sigma Aldrich, St Louis, MO). The frog was submitted to the University of Illinois Veterinary Diagnostic Laboratory for necropsy examination.

On gross necropsy, the frog was found to be in poor nutritional condition. The frog weighed 86.3 g, and intraceolomic fat pads were absent. No other significant gross abnormalities were observed.

Aerobic bacterial culture of the spleen yielded no pathogens. Samples of the spleen were analyzed by PCR using genus-specific mycobacterial primers, ITSF and myocm 2.[12,13] Genomic DNA was extracted from the spleen by using a purification kit (no. 947057, One-For-All Vet Purification Kit, Qiagen, Venlo, Limburg, The Netherlands) and an automated processor (BioSprint processor, Qiagen). The 25-µL PCR reaction volume consisted of 2.5 µL 10× buffer A (Thermo Fisher Scientific, Waltham, MA), 2.5 µL 2 nM dNTP, 10 µM each ITSF and mycom 2 primers, 0.25 µL 5U/µL *Taq* polymerase (Invitrogen), 12.25 µL sterile water, and 2.5 µL template DNA. Thermocycler conditions comprised 94 °C denaturation for 5 min followed by 29 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, with a final extension for 10 min; then the reaction was held at 4 °C. Electrophoresis of a positive amplification product on a 1% to 2% agarose gel yields a 270- to 360-bp band. No amplification of *Mycobacterium* spp. DNA from the frog spleen was detected.

Formalin-fixed samples were collected, and samples were paraffin-embedded, routinely processed, sectioned at 4 to 5 µm, and stained with hematoxylin and eosin. Tissue sections examined included lungs, liver, kidneys, spleen, heart, small intestine, pancreas, ovaries, stomach, small and large intestine, skin, and skeletal

Received: 25 Apr 2014. Revision requested: 31 May 2014. Accepted: 30 Jul 2014.
[1]Veterinary Diagnostic Laboratory, [2]Division of Animal Resources, Illinois, and [3]Department of Pathobiology, University of Illinois, Urbana, Illinois.
[*]Corresponding author. Email: awstern@illinois.edu

muscle. The pancreas was nearly completely effaced by a neoplastic cell population arranged in packets and supported by a thin fibrovascular stroma (Figure 1). Neoplastic cells were polyhedral, had distinct cell margins, frequent high nuclear:cytoplasmic ratio, and eosinophilic and wispy cytoplasm often containing variably sized eosinophilic globules. Nuclei were round, with stippled chromatin and 1 to 3 nucleoli. Neoplastic cells exhibited moderate anisocytosis and anisokaryosis. There were 1 to 3 mitotic figures per random 400× field. Scattered single-cell necrosis of the neoplastic cells and rare remnant pancreatic acinar cells were observed. Histochemical staining with Masson trichome was performed and there was collagen-positive (blue) staining of the fibrous stroma separating the packets of neoplastic cells (Figure 2).

Immunohistochemical staining with a panel of antibodies, including cytokeratin (AE1/AE3), insulin, glucagon, and synaptophysin, was performed. Given the limited amount of information in the literature about the suitability of amphibian tissue for immunohistochemical staining, we used nonneoplastic *Xenopus* frog pancreas for positive-control tissues to evaluate the staining of the target (that is, neoplastic) tissue. Immunohistochemical staining for synaptophysin, insulin, and glucagon were negative both in the *Xenopus* frog control tissue and neoplastic cell population. Immunohistochemical staining with cytokeratin was performed and was positive in both the control tissues and neoplastic cell population. Approximately 10% of neoplastic cells had positive cytoplasmic immunoreactivity for cytokeratin.

The histomorphology of the neoplasm was consistent with a pancreatic carcinoma, which likely arose from pancreatic islet cells because the formation of packets of cells is typical of islet cell tumors. Immunohistochemical staining was performed in an attempt to further characterize the neoplastic cells. However, the control tissues did not exhibit appropriate immunoreactivity, and the results of the synaptophysin, insulin, and glucagon stains could not be interpreted. Metastatic disease was not identified in this animal. Although there is a history of mycobacterial infections in this colony of frogs, there was no evidence of mycobacteriosis in this particular animal.

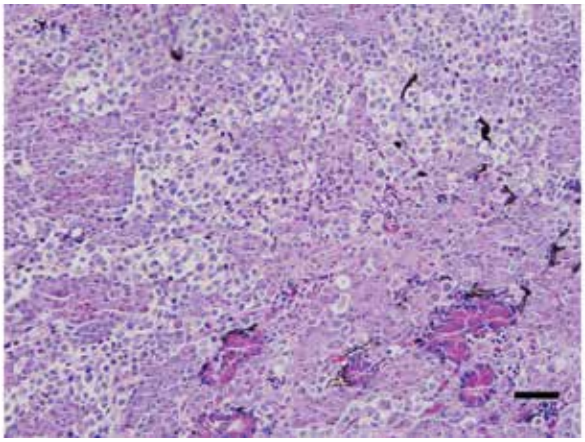

Figure 1. Histopathology of the pancreas. Note that the pancreas is almost completely effaced by the neoplastic cell population. Hematoxylin and eosin stain; bar, 20 µm.

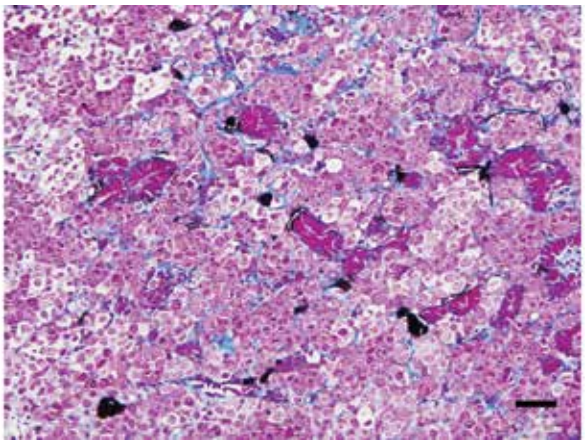

Figure 2. Histopathology of the pancreas. Note the positive (blue, collagen) staining of the supporting stroma. Masson trichrome stain; bar, 20 µm.

Discussion

Causes of weight loss in *X. laevis* include both infectious and noninfectious diseases (neoplasia and husbandry-related issues). Infectious agents include bacteria (*Mycobacterium* spp., *Flavobacterium* spp., *Salmonella* spp., and *Aeromonas* spp.), parasites (protozoans, nematodes, trematodes, and cestodes), and fungi.[7,11] The index animal did not have any dermatologic signs of an infectious disease, such as skin sloughing, ulceration, petechiation, and nodules, or general clinical signs, such as lethargy or coelomic effusion.[7,11] In this case, postmortem findings did not reveal lesions consistent with a parasitic, bacterial, or fungal etiology. Factors such as stress, poor husbandry, and neoplasia can also lead to poor body condition. Stress could have been a factor in the current case, but the tank occupancy was within facility guidelines and standard operating procedures for the husbandry of captive aquatic frogs (3.79 L [1 gal] water per adult frog). In addition, water-quality parameters were within acceptable ranges.

This colony of *X. laevis* had a history of mycobacterial infections. For the majority of the clinical cases, speciation of the mycobacterial amplicon sequence was not included as part of the diagnostic testing. For those cases in which it was performed, *M. marinum* was isolated from 2 frogs, both *M. ulcerans* and *M. marinum* were isolated from 1 animal, and *M. linflandii* was obtained from 1 animal. Clinical signs varied among these cases and included coloemic distention, lethargy, and skin lesions including petechiation, erythema, sloughing, and ulceration.

Although neoplastic diseases are reported in amphibians, they are considered to be uncommon occurrences.[3] A majority of neoplasms in amphibians have been reported from captive specimens, although wild specimens have also been. Neoplasia of the pancreas has rarely been reported in frogs. In one report in pond frogs of the *Pelophylax* (previously *Rana*) genus, neoplastic cells of pancreatic carcinomas contained both exocrine and endocrine secretion granules.[9] In one case series,[9] 11 pancreatic carcinomas were stained for insulin, somatostatin, and glucagon; 4 neoplasms were positive for insulin, 8 neoplasms were positive for somatostatin, and all 11 were negative for glucagon. In addition, all pancreatic carcinomas had mature C-type retrovirus particles in the extracellular spaces, but these particles were not present in the nonneoplastic pancreatic tissue.[9] In comparison to this previous report, we have examined approximately 20 frogs from the research laboratory where the current frog was housed, and no

pancreatic neoplasms or any other neoplastic processes have been identified in any animal examined.

In *Xenopus*, neoplastic processes are reported infrequently. Hepatomas perhaps occur most often, with 5% of *Xenopus* frogs affected per year in one colony.[5] In addition, ovarian dysgerminoma has been reported in several *Xenopus* frogs.[5] The neoplastic cells of dysgerminomas are histomorphologically similar to those of seminomas in males. Ovarian dysgerminoma has also been reported to occur in mountain chicken frogs (*Leptodactylus fallax*).[4]

X. laevis that develop nonspecific signs of illness such as poor body condition require a thorough assessment and diagnostic testing. In the laboratory setting, it is important to rule in and rule out infectious diseases in addition to evaluating and identifying other potential disease processes, such as neoplasia. The frog we describe here had a pancreatic carcinoma; to our knowledge, this is the first report of this neoplasm in *Xenopus laevis*.

References

1. **Balls M.** 1962. Spontaneous neoplasms in amphibia: a review and descriptions of 6 new cases. Cancer Res **22:**1142–1154.
2. **Cheong SW, Fukui A, Asashima M, Pfeifer CJ.** 2000. Spontaneous thyroid-containing teratoma associated with impaired development in the African clawed frog, *Xenopus laevis*. J Comp Pathol **123:**110–118.
3. **Densmore CL, Green DE.** 2007. Diseases of amphibians. ILAR J **48:**235–254.
4. **Fitzgerald SD, Duncan AE, Tabaka C, Garner MM, Dieter A, Kiupel M.** 2007. Ovarian dysgerminoma in 2 mountain chicken frogs (Leptodactylus fallax). J Zoo Wildl Med **38:**150–153.
5. **Goyos A, Robert J.** 2009. Tumorigenesis and antitumor immune responses in *Xenopus*. Front Biosci (Landmark Ed) **14:**167–176.
6. **Green DE, Harshbarger JC.** 2001. Spontaneous neoplasms in amphibia, p 335–400. In: Write KM, Whitaker BR, editors. Amphibian medicine and captive husbandry. Malabar (FL): Krieger Publishing.
7. **Green SL.** 2010. The laboratory *Xenopus* spp. Boca Raton (FL): CRC Press.
8. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
9. **Masahito P, Nishioka M, Ueda H, Kato Y, Yamazaki I, Nomura K, Sugano H, Kitagawa T.** 1995. Frequent development of pancreatic carcinomas in the *Rana nigromaculata* group. Cancer Res **55:**3781–3784.
10. **Meyer-Rochow VB, Asashima M, Moro SD.** 1991. Nephroblastoma in the clawed frog *Xenopus laevis*. J Exp Anim Sci **34:**225–228.
11. **O'Rourke DP, Schultz TW.** 2002. Biology and diseases of amphibians, p793–826. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory animal medicine, 2nd ed. San Diego (CA): Academic Press.
12. **Park H, Jang H, Kim C, Chung B, Chang CL, Park SK, Song S.** 2000. Detection and identification of mycobacteria by amplification of the internal transcribed spacer regions with genus- and species-specific PCR primers. J Clin Microbiol **38:**4080–4085.
13. **Park H, Jang H, Kim C, Chung B, Chang CL, Park SK, Song S.** 2001. Detection and identification of mycobacteria by amplification of the internal transcribed spacer regions with genus- and species-specific PCR primers. J Clin Microbiol **39:**828.
14. **Stacy BA, Parker JM.** 2004. Amphibian oncology. Vet Clin North Am Exot Anim Pract **7:**673–695.
15. **Tsonis PA, Rio-Tsonis KD.** 1988. Spontaneous neoplasms in Amphibia. Tumour Biol **9:**221–224.