

Mature congenital intraneural teratoma in cerebellum of pig

Adolfo Soto-Domínguez^{1*}, Humberto Rodríguez-Rocha^{1*}, Aracely García-García¹,
Odila Saucedo-Cárdenas^{1,2}, Luis E. Rodríguez-Tovar³ and Uziel Castillo-Velázquez³

¹Department of Histology, School of Medicine, Autonomous University of Nuevo León, ²Northeastern Biomedical Research Center (CIBIN), IMSS, Monterrey and ³Department of Veterinary Immunology, School of Veterinary Medicine and Zootechnics, Autonomous University of Nuevo León, México.

*Equal contribution

Summary. The biological behavior of teratomas depends on several interdependent clinical and epidemiological variables such as age at diagnosis, sex, tumor microenvironment, and tumor morphology, among others. All these variables are correlated to different cytogenetic and molecular aberrations (Harms et al., 2006). There are null reports of teratomas in pigs. The aim of this study was to characterize the tissues present in a mature congenital intraneural teratoma in the cerebellum area of a Landrace female pig of 6-7 weeks old. In this study, tissue control samples were used to validate each staining method. Sections from the teratoma showed normal histology of the cerebellum, including rounded Purkinje neurons with abundant cytoplasm, euchromatic nuclei, and prominent nucleoli; glial cells with a scarce amount of cytoplasm and small and highly basophile-nuclei (compact chromatin) and axonal tracts (white matter). Interestingly, we also observed areas with tissues different from the nervous tissue, including bundles of well-defined skeletal muscle fibers with a striated pattern and peripheral nuclei; hyaline cartilage plaques, with prominent presence of chondrocytes in their lagoons forming isogenous groups surrounded by a territorial and interterritorial matrix; trabeculated bone tissue; and adipocytes, which are ring-shaped cells with peripheral flattened nuclei, as a result

of the presence of a central large lipid droplet. To our knowledge, this study is the first to describe a congenital intraneural mature teratoma in the cerebellum of a pig.

Key words: Teratoma, Pig, Cerebellum, Intraneural

Introduction

Teratomas are uncommon tumors containing elements that originate from more than one germ-cell layer, foreign to the organ in which they arise, and show independent growth, which is composed of a mixture of different tissue types representative of ectoderm, endoderm, and mesoderm (Nielsen and Kennedy, 1990). They can have mature (well-differentiated) or immature histological components (Horowitz and Hall, 1991). They belong to the family of germ-cell tumors, which also includes germinoma, embryonic carcinoma, endodermal sinus tumor, and choriocarcinoma, each of which represents the malignant transformation of an embryonic tissue stage (Horowitz and Hall, 1991; Jennings et al., 1985). Teratomas and germ-cell tumors, in general, are mostly found in the gonads but sometimes occur in the neck along the midline of the brain, generally involving the suprasellar, thalamic, or pineal regions (Cordy, 1984; Jennings et al., 1985; Kountakis et al., 1994). In veterinary medicine, intracranial germ-cell tumors have been reported mostly in dogs (Patnaik and Nafe, 1980; Valentine et al., 1988; Horowitz and Hall, 1991; Nyska et al., 1993), but also in duck (Homer and Riggs, 1991), rat (Reindel et al., 1996), chicken (Jones,

1964), horse (Mettam, 1915), and rabbit (Bishop, 1978). The affected animals were generally juveniles or young adults.

Histologically, teratomas are classified as mature or immature based on the presence of differentiated tissues or immature elements, respectively, and are anatomically divided into gonadal or extragonadal. Most teratomas described in domestic animals and humans (Jennings et al., 1985; Horowitz and Hall, 1991) are gonadal. Extragonadal teratomas have been described in horse placenta (*Equus caballus*), adrenal gland of ferrets (*Mustela putorius furo*), the umbilical cord of a giraffe (*Giraffa camelopardalis reticulata*), skin of a roe deer (*Capreolus capreolus*), and kidney of a llama (*Lama glama*). Additionally, extragonadal intracranial teratomas were reported in rabbit (*Oryctolagus cuniculus*), dog (*Canis lupus familiaris*), cat (*Felis catus*), and alpaca (*Vicugna pacos*) (Headley et al., 2016). However, there are null reports of teratomas in pigs. The aim of this study was to characterize the tissues present in a mature congenital intraneural teratoma in the cerebellum area of a Landrace female pig of 6-7 weeks old. Histological, histochemical and immunohistochemical methods were applied.

Material and methods

Case presentation

A landrace female pig of 6-7 weeks and 15 kg of weight was obtained from the 4th litter by artificial insemination. This pig did not show signs of disease on clinical examination. This pig was obtained from a commercial meat supply farm in the community of Los Ramones, Nuevo Leon, Mexico. This place is located at the following coordinates: 25° 42'00" N 99° 37'00" W.

Tissue samples collection

The initial objective was to obtain biological samples to elaborate a collection of histological sections for didactic purposes. However, the presence of a teratoma was observed in the cerebellar area when the encephalous samples were analyzed, so we decided to characterize the mature tissues present in the teratoma. After the acquisition, the animal was maintained in the facilities of the School of Veterinary Medicine and Zootechnics, of the UANL. The pig was maintained on a standard diet, *ad libitum* water access, and light and dark cycles of 12 h. Management of the animal was carried out according to the International Guidelines on the Appropriate Use of Experimental Animals, and according to Mexican Norm NOM-062-ZOO-1999 on the Technical Specifications for Production, Care and Use of Laboratory Animals.

Before the euthanasia, the animal was anesthetized with a dose of 1.1 mg/body weight of ketamine via intramuscular. The pig was euthanized by cervical dislocation and tissue samples were collected.

Morphological analysis

Samples were rinsed with phosphate buffered saline (PBS) 1x, pH 7.2-7.4, and then fixed by immersion in 4% formaldehyde in PBS 1x solution for 24 h. Samples were then processed by conventional histological technique and embedded in paraffin blocks. Histological sections of 4 μ m were obtained and stained with hematoxylin and eosin (H&E), or Masson's trichrome staining for histological analysis.

For histochemical evaluation, sections of 7 μ m were tested with Klüver–Barrera method to evaluate the myelin within the nerve tracts (Klüver and Barrera, 1953).

Histological slices of 4 μ m were analyzed with the Periodic Acid-Schiff (PAS) staining to identify polysaccharide complexes present in the extracellular matrix of cartilage and muscle fibers.

Additionally, the von Kossa staining for calcium detection was applied. This method is used to visualize calcium deposits in paraffin sections, where tissues are treated with a silver nitrate solution, which replaces the calcium, and therefore, can be visualized as metallic silver deposits (von Kossa, 1901).

Immunostaining analysis was carried out to identify the tissues present in the samples with specific antibodies. This was performed in 4 μ m sections. Skeletal muscle fibers were identified with anti-skeletal muscle actin (SMA) (1:200, HHF35, Dako Cytomation Inc[®]); bone tissue with anti-collagen I (COL I) (1:200, ab23446, Abcam[®]) and osteoblast with anti-parathyroid hormone receptor (PTHr) (1:400, C313515, LSBio LifeSpan BioSciences, inc); anti-collagen II (COL II) (1:200, ab34712, Abcam[®]) was used to detect cartilage; anti-cytokeratins AE1/AE3 (1:200, M3515, Dako) to identify epithelial cells and anti-myelin basic protein (MBP) (1: 500, Dako) for nervous tissue.

Mouse and rabbit specific HRP/DAB (ABC) detection IHC kit (ab64264, Abcam) was used. Positivity was identified with 3, 3'-diaminobenzidine (DAB), and nuclei were contrasted with Gill's hematoxylin.

Sections of the tongue, decalcified bone, hyaline cartilage, palatine tonsil, and cerebellum were used as positive controls, respectively. Samples were analyzed by light microscopy.

Results

Abnormal tissue growth in the cerebellum of pig

Sections from the cerebellum were stained with H&E (Fig.1) and displayed normal histology of the cerebellum, including rounded Purkinje neurons with abundant cytoplasm and euchromatic nuclei with prominent nucleoli. Smaller nuclei with compact chromatin and few cytoplasm (corresponding to glial cells) and tracts of axons of white matter were also observed (Fig. 1). Interestingly, we observed areas with

Teratoma in the cerebellum of pig

different components from the nervous tissue such as bundles of well-defined skeletal muscle fibers with a striated pattern and nuclei in the periphery. Plaques of hyaline cartilage were also observed in which chondrocytes are prominent in their lagoons and surrounded by a territorial and interterritorial matrix, as well as isogenous groups. In these same areas, trabeculae of bone tissue were observed. Finally, we found adipocytes, which are ring-shaped cells with peripheral flattened nuclei, as a result of the presence of a central large lipid droplet. (Fig. 2B-D). Tissue control samples were used to validate each staining method: hyaline cartilage (Fig. 2A,M), tongue (Fig. 2E), lung granuloma (Fig. 2I) and brain (Fig. 2Q).

Additionally, collagen fibers and muscle fibers were more evident in teratoma areas with the Masson's trichrome staining (Fig. 2F-H).

Calcium associated with trabecular bone was observed as black deposits of silver impregnation (Fig. 2J-L).

Plaques of cartilage and muscle cells showed an intense magenta color with the PAS staining, which evidenced the presence of glucosaminoglycans and proteoglycans in the extracellular matrix (Fig. 2N-P).

Finally, in sections stained with the Klüver-Barrera method, the cerebellum displayed fascicles of axons with uniform blue-stained myelin, organized neuropil and neurons with normal appearance (Fig. 2R-T). Therefore,

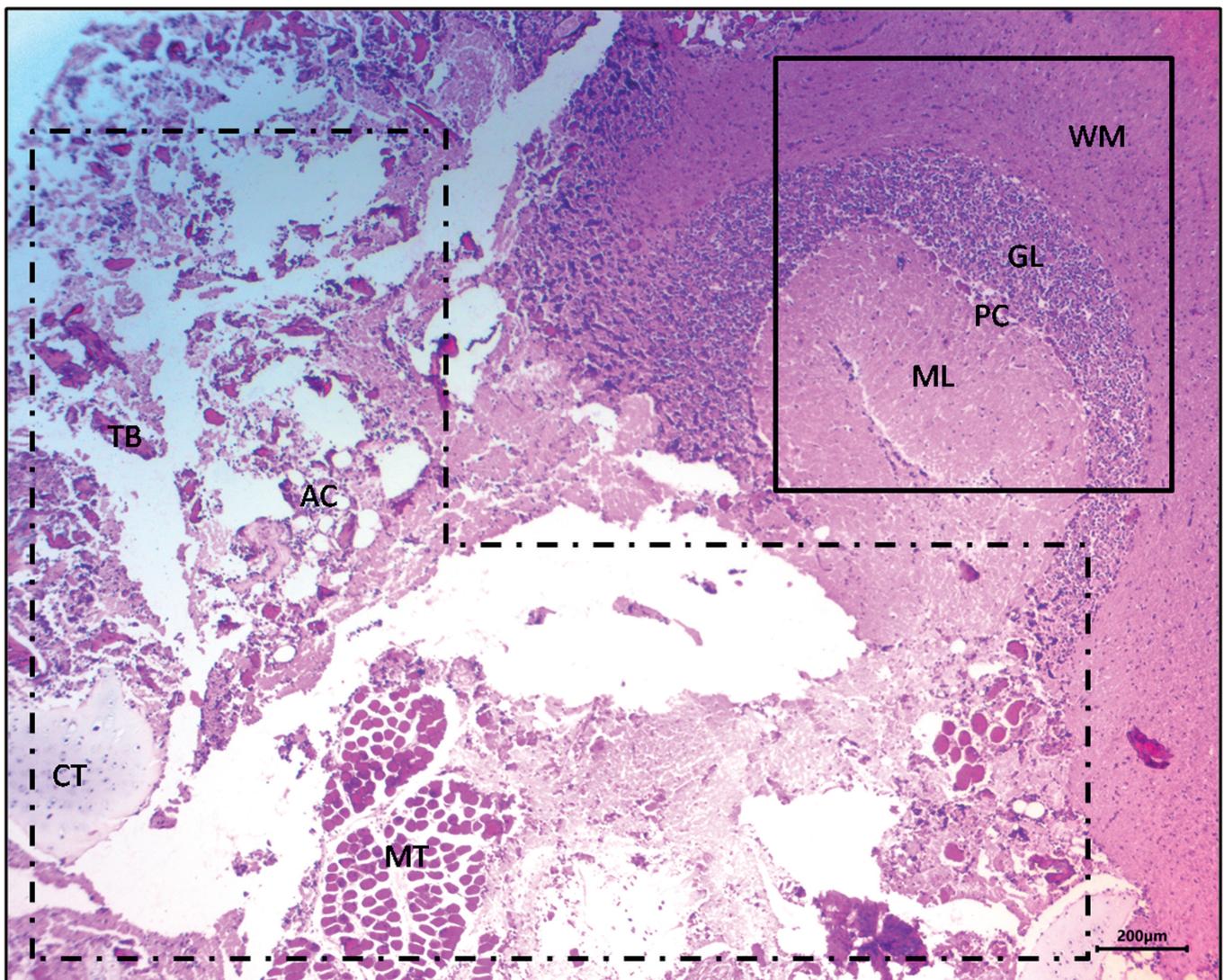


Fig. 1. Nervous tissue sample from the cerebellum of a pig. Normal nervous tissue from cerebellum area is enclosed in the continuous line square, showing the molecular (ML), Purkinje cells (PC) and granule (GL) layers, as well as the white matter (WM). Mature tissues present in teratoma area are enclosed in discontinuous line square, showing cartilage (CT), skeletal muscle (MT), trabecular bone (TB) and adipose cells (AC). HE staining. Scale bar: 200 μm .

Teratoma in the cerebellum of pig

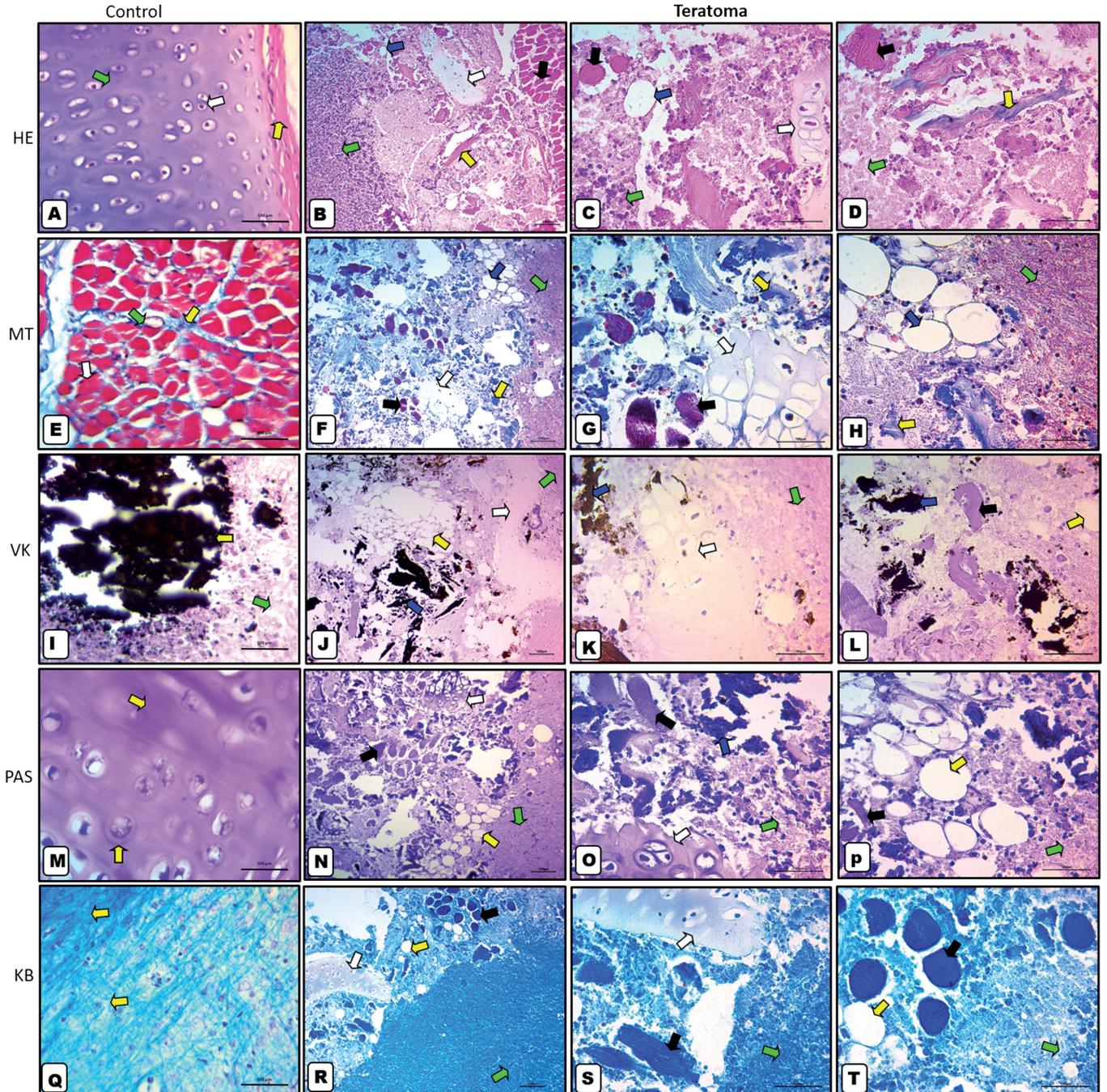


Fig. 2. Morphological identification of abnormal tissue in the cerebellum of a pig. **A.** Hyaline cartilage shows perichondrium (yellow arrow), chondrocyte (white arrow), and matrix (green arrow). **B-D.** Images of teratoma showing skeletal muscle (black arrows), cartilage (white arrows), trabecular bone (yellow arrows), adipose cells (blue arrows), and nervous tissue (green arrows). **E.** Presence of skeletal muscle cells in tongue (white arrow), collagen fibers (yellow arrow), and blood vessel (green arrow). **F-H.** Micrographs of teratoma that show cartilage (white arrows), collagen fibers in trabecular bone in blue color (yellow arrows), muscle cells in red stain (black arrows), adipose cells (blue arrows), and nervous tissue (green arrows). **(I)** Granuloma with calcified center (yellow arrow), and lung tissue (green arrow). **J, K, and L.** Teratoma with bone positive areas (blue arrows), cartilage (white arrows), adipose cells (yellow arrow), muscle cells (black arrows), and nervous tissue (green arrows). **M.** Hyaline cartilage that shows PAS positive areas (yellow arrows). **N, O, and P.** Teratoma PAS positive in muscle cells (black arrows) and cartilage (white arrows), and PAS negative in trabecular bone (blue arrows), adipocytes (yellow arrows) and nervous tissue (green arrows). **Q.** Myeline positive in white matter in the brain (yellow arrows). **R, S, and T.** Micrographs of teratoma that show myeline in nervous tissue (green arrows). Cartilage (white arrows), adipocytes (yellow arrows) and muscle (black arrows). **A-D.** Hematoxylin and Eosin (HE). **E-H.** Masson's trichrome stain (MT). **I-L.** Von Kossa staining (VK). **M-P.** Periodic acid Schiff (PAS). **Q-T.** Klüver-Barrera staining (KV). Scale bars: 100 μm .

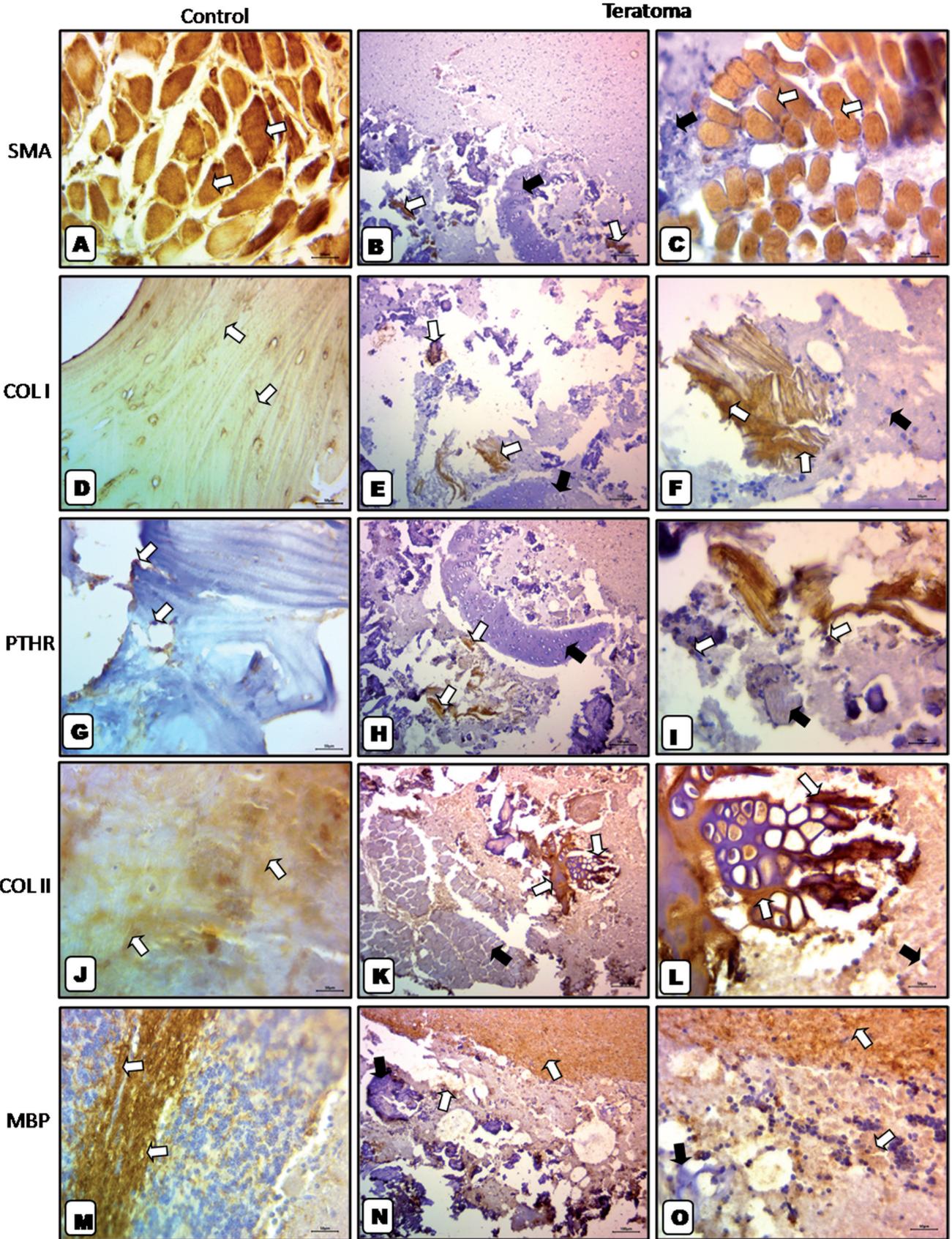


Fig. 3. Specific identification of abnormal tissue in the cerebellum of a pig. Specific antibodies were used to identify abnormal tissues in cerebellum of pig: anti-skeletal muscle actin (SMA) for muscle fibers (B-C), anti-collagen I (COL I) for bone tissue (E-F), anti-parathyroid hormone receptor (PTHR) for osteoblast (H-I), anti-collagen II (COL II) for cartilage (K-L), and anti-myelin basic protein (MBP) for nervous tissue detection (N-O). Positive results for each antibody (white arrows), negative results (black arrows). Method control samples included tongue (A), decalcified bone (D and G), hyaline cartilage (J) and cerebellum (M). Scale bars: B, E, H, K, N, 100 μ m; A, D, G, J, M, C, F, I, L, O, 50 μ m.

abnormal tissue growth in the cerebellum of the pig was evidenced by histological staining corroborating the teratoma presence.

Identification of abnormal tissue in the cerebellum of pig

In order to determine the teratoma components, we performed their immunohistochemical identification using specific antibodies, confirming the presence of skeletal muscle fibers (Fig. 3B,C), bone tissue (Fig. 3E, F, H and I), cartilage (Fig. 3K and L). The white matter of cerebellum is also shown (Fig. 3N,O). Negative results were observed for AE1/AE3 cytokeratins (data not shown).

Tissue control samples were used to validate the immunolabeling method: tongue (Fig. 3A), decalcified bone (Fig. 3D and G), hyaline cartilage (Fig. 3J) and cerebellum (Fig. 3M).

Taken together, these results demonstrated, for the first time, the presence of a mature congenital intraneural teratoma in the cerebellum of a pig.

Discussion

This study is the first to describe a mature congenital intraneural teratoma in a pig cerebellum. We characterized the mature tissues present such as striated skeletal muscle fibers, cartilage, trabecular bone and possible adipose tissue.

Teratomas are tumors that contain elements derived from more than one of the three embryonic blastodermal leaves. As a result, tissue components are often foreign to the anatomical location of the tumor. The cause of teratomas remains unclear and may vary depending on the location, gonadal or extragonadal. Two histogenetic hypotheses of the extragonadal teratomas are considered: a) origin in residual germ cells in the different levels of their normal migration during inbreeding, and b) development from totipotential embryonic somatic cells not modulated by the mechanisms of morphogenic regulation (Gonzalez-Crussi, 1982).

Compared with other tumors, teratomas have a variety of histological mature tissues rather than a proliferation of neoplastic cells. They are composed of heterotypic tissues, including epidermis, nerve tissue or mature cartilage. However, they also contain nonspecific tissue, such as lymphoid or fibrous stroma. Classical theories of the origin of teratomas include incomplete twins, sequestration of totipotential blastomeres or stem cells that degenerate to neoplastic proliferation, depression of totipotential genetic information to the nucleus of somatic cells, and parthenogenic development of germ cells (Vortmeyer et al., 1999). Histologically, teratomas are divided into three groups: 1) mature, 2) immature, and 3) monodermal or very specialized (Cotran et al., 2005). Due to the incidence of teratomas, the origin from totipotential stem cells was postulated decades ago, using polymorphisms and enzymes as well as chromosome studies. Linder et al. (1975)

demonstrated that teratomas are homozygous for chromosome polymorphisms, while the other non-teratomatous germ cell tumors are heterozygous. Compared with other tumors, teratomas have a histological feature composed of a variety of tissues architecturally mature. Immature teratomas are made up of embryonic-looking tissues. They may be derived from hair, cartilaginous material, bone, calcified areas, and tissue in differentiation pathways to glands, nerves, and other structures, hyaline bodies being microscopically found. The mature teratoma or dermoid cyst represents the most common neoplasm. They show a thin wall covered by a wrinkled opaque greyish-white epidermis, from which departs hairy stems, dental structures, and calcified areas, as well as cartilage, bone, thyroid tissue and other structures. In rare cases, a solid teratoma is composed entirely of heterogeneous benign-looking mixtures derived from the three germ layers, which probably have the same histogenic origin as the dermoid cysts, but lack the predominance of ectodermal tissue. They are difficult to distinguish from malignant teratomas that are generally solid (Gómez-Monterrosas et al., 2006). There is no doubt that this neoplasm is a true tumor displaying progressive growth and composed of multiple tissues foreign to the part in which it was located. Mitotic figures were not seen, but the overall impression was that of live tissue in the active process of growing active secretory glands and forming cysts. The islands of cartilage were complete, with fibrous and chondrogenic perichondrium actively differentiating and cartilage developing by appositional and interstitial growth. Outside of the ovary and testicle, teratomas in the pig have not been found. So far as can be ascertained, this is the first report of a primary teratoma in the cerebellum of a pig.

In this study, we used control tissue samples to validate the staining method and immunohistochemical identification, such as hyaline cartilage, to validate the presence of cartilage in the teratoma, as well as using a lingual tissue, pulmonary granuloma and brain to validate the techniques employed. Structures corresponding to cerebellar tissue were observed when performing H&E staining. However, it was also possible to identify structures outside this tissue such as muscle fibers, specifically skeletal muscle, hyaline cartilage, specifically chondrocytes, and trabeculae of bone tissue and structures compatible with adipocytes, but because of the inclusion method used it was not possible to identify these structures. These structures outside the cerebellar tissue were identified by histological techniques such as Masson's trichrome, silver impregnation and PAS histochemical staining and finally Klüver and Barrera to identify normal-looking cerebellar tissue. Immunoblotting was also used to identify the cell lineage, such as actin for striated muscle, collagen I for bone tissue and collagen type II for osseous and cartilaginous tissue, respectively. One important point is the fact of observing positivity for PTHR since it is associated with regulation of cell proliferation and cell

Teratoma in the cerebellum of pig

differentiation and apoptosis of multiple tissues, which assures us the viability of these structures and their degree of differentiation.

Acknowledgments. We acknowledge technical assistance from MVZ Luis I. Botello-Soto and Ivan A. Mesta-Rodríguez. We thank Stefanie G. Ibarra S., Dennis A. Patiño C., Adrián E. Hernández C., and Christian A. Gómez T., for their technical participation (immunolabeling) in the PROVERICYT (Programa de Verano de la Investigación Científica y Tecnológica), of the UANL.

Conflict of interest. The authors declare that there is no conflict of interest regarding the publication of this article.

References

- Bishop L. (1978). Intracranial teratoma in a domestic rabbit. *Vet. Pathol.* 15, 525-530
- Cordy D.R. (1984). Intracranial germinoma in a dog. *Vet. Pathol.* 21, 357-358.
- Cotran R.S., Kumar V. and Robbins S.L. (2005). *Patología estructural y funcional*. 7ª edición. McGraw-Hill. pp 11-18.
- Gómez-Monterrosas O., Flores-García L.E., Gómez-Garza G. Pichardo-Bahena R., Pantoja J.P. and Méndez-Sánchez N. (2006). Teratoma maduro de ovario como causa de abdomen agudo. *Reporte de un caso. Médica Sur Méjico*, 13, 178-181.
- Gonzalez-Crussi F. (1982). Extragenital teratomas. *Atlas of tumor pathology* pp 77-94.
- Harms D., Zahn S., Göbel U., and Schneider D.T. (2006). Pathology and molecular biology of teratomas in childhood and adolescence. *Klinische Pädiatrie* 218, 296-302.
- Headley S.A., Costa T.C., Marcasso R.A., Hilst C.L., Bracarense A.P. and Di Santis G.W. (2016). Congenital Mature Intracranial Teratoma in a Pampas Deer (*Ozotoceros bezoarticus*) in Brazil. *J. wildl. Dis.* 52, 749-752.
- Homer B.L. and Riggs M.W. (1991). Cranial teratomas in two domestic ducks (*Anas platyrhynchos domesticus*). *Avian Dis.* 35, 994-998.
- Horowitz M.B. and Hall W.A. (1991). Central nervous system germinomas. *Arch. Neurol.* 48, 652-657.
- Jennings M.T., Gelman R. and Hochberg F. (1985). Intracranial germ cell tumors: natural history and pathogenesis (review). *J. Neurosurg.* 63, 155-167.
- Jones L.D. (1964). Avian cerebellar teratoma. *Avian Dis.* 8, 580-584.
- Klüver H. and Barrera E. (1953). A method for the combined staining of cells and fibers in the nervous system. *J. Neuropathol. Exp. Neurol.* 12, 400-403.
- Kountakis S.E., Minotti A.M., Maillard A. and Stiernberg C.M. (1994). Teratomas of the head and neck. *Am. J. Otolaryngol.* 15, 292-296.
- Linder D., Hecht F., McCaw B.K. and Campbell J.R. (1975). Origin of extragenital teratomas and endodermal sinus tumors. *Nature* 254, 597-598.
- Mettam A.E. (1915). Embryoma of the brain of the horse. *J. Comp. Pathol.* 28, 52-53.
- Nielsen S.W. and Kennedy P.C. (1990). Tumors of the genital systems. In: *Tumors in domestic animals*. 3rd ed. Moulton J.E. (ed). University of California Press, Berkeley, CA pp 489-490.
- Nyska A., Harmelin A., Baneth G., Yakobson B., Shmuel P., Orgad U. and Warner T. (1993). Suprasellar differentiated germ cell tumor in a male dog. *J. Vet. Diagn. Invest.* 5, 462-467.
- Patnaik A.K. and Nafe L.A. (1980). Intracranial teratocarcinoma in a dog. *Vet. Pathol.* 17, 764-769.
- Reindel J., Bobrowski W., Gough A. and Anderson J. (1996). Malignant intracranial teratoma in a juvenile Wistar rat. *Vet. Pathol.* 33, 462-465.
- Valentine B.A., Summers B.A., de Lahunta A., White C.L. 3rd. And Kuhajda F.P. (1988). Suprasellar germ cell tumors in the dog: a report of five cases and review of the literature. *Acta Neuropathol.* 76, 94-100.
- Von Kossa J. (1901). Ueber die in organismus kunstlich erzeugbaren verkaikungen. *Beit Path. Anat.* 29, 163.
- Vortmeyer A.O., Devouassoux-Shisheboran M., Li G., Mohr V., Tavassoli F. and Zhuang Z. (1999). Microdissection-Based analysis of mature ovarian teratoma. *Am. J. Pathol.* 154, 987-991.

Accepted September 5, 2018