Case Report

Management of multiple intracranial granular cell tumors in a golden retiever

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Abstract

A 9-year-old, neutered female Golden Retriever was presented with generalized seizures, abnormal mentation and ataxia. Magnetic resonance imaging revealed five separated masses at the left dorsal meninges, caudodorsal cerebellum, right cerebral cortex, olfactory lobe and right occipital lobe. Right rostrotentorial craniectomy was performed to debulk the tumor at the right cerebral cortex. On the basis of histopathological evaluation, the mass was diagnosed as GCTs. Immunohistochemistry of the mass demonstrated immunopositivity for vimentin and immunonegativity for GFAP, NSE, Desmin, S-100, CK14, CK8/18, CK19, and Ki67. Postoperative neurological deficits were resolved, but six months later neurological signs recurred with higher frequency of seizures and more severe ataxia than previously noted. This time suboccipital craniectomy was performed to remove the mass at the caudodorsal cerebellum. Histopathological evaluation of the mass confirmed GCTs. The dog did not survive and died unexpectedly during recovery period after the second operation.

Keywords: canine, brain, surgery, granular cell tumor

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Introduction

Granular cell tumors (GCTs) are rare tumors in both humans and animals that may occur within or outside the nervous system. They are the most common intracranial neoplasms in rats (Yoshida et al., 1997) and have also been reported in other species including dog, cat, bird and horse (Patnaik, 1993; Higgins et al., 2001; Mandara et al., 2006; Mishra et al., 2012). There are several reports of canine GCTs in the meninges (Higgins et al., 2001; Mishra et al., 2012). Based on ultrastructural and immunohistochemical studies, the meningeal cells have been thought to be an origin of GCTs in rat (Yoshida et al., 1997). Moreover, histological evaluation of GCTs in dogs and a cat has suggested these tumors to be of meningeal origin (Higgins et al., 2001; Mandara et al., 2006). Although there is minimal variation in the cell morphology of GCTs, their histogenesis mainly remains unclear. The purpose of this report, therefore, was to describe neuroimaging, and clinical, histological and immunohistochemical findings of canine multiple intracranial GCTs.

History and Clinical findings

A 9-year-old neutered female Golden Retriever was referred to Kasetsart University Veterinary Teaching Hospital with the main complaints of generalized seizures, abnormal mentation and ataxia for 3 months. Clinical signs started to show and progress almost 3 weeks before the dog was presented to the hospital with right circling at postictal phase. Neurological examination revealed disorientation, right circling, and proprioceptive deficit on the left side. Spinal reflexes, however, were normal. Cranial nerve examination demonstrated absent menace response and loss of vision on the left eye. The palpebral and pupillary light reflexes were normal in both eyes. All other aspects of the neurological examination were within normal limits. Based on the neurological examination and history of seizures, the neuroanatomic diagnosis was consistent with the right forebrain lesion. Differential diagnosis considered after initial examination included intracranial neoplasia and inflammatory disease. Complete blood count and blood chemistry profiles were within normal limits. Thoracic radiograph revealed slight changes in the bronchial walls consistent with aging without evidence of metastatic disease.

Magnetic resonance imaging (MRI) of the brain was performed using 1.5 tesla unit. The following pulse sequences were obtained in the transverse and sagittal planes: T1-weighted (T1W), T2-weighted (T2W), and T2W FLAIR. Images were obtained in the transverse, dorsal, and sagittal planes after intravenous administration of gadolinium. MRI showed that there were five separated masses at the left dorsal meninges, caudodorsal cerebellum, right cerebral cortex, olfactory lobe, and right occipital lobe (Figure 1). On T1W, obtained following the intravenous administration of contrast media, the meninges of dorsal aspects of the right cerebral cortex and olfactory lobe displayed heterogeneous enhancement. In addition to the meningeal enhancement, there was a marked enlargement, a hyperintensity mass at the right cerebral cortex resulting in compression of the right lateral ventricle as revealed by severe deviation of the falx cerebri to the left. Moreover, there was caudodorsal compression of the cerebellum.

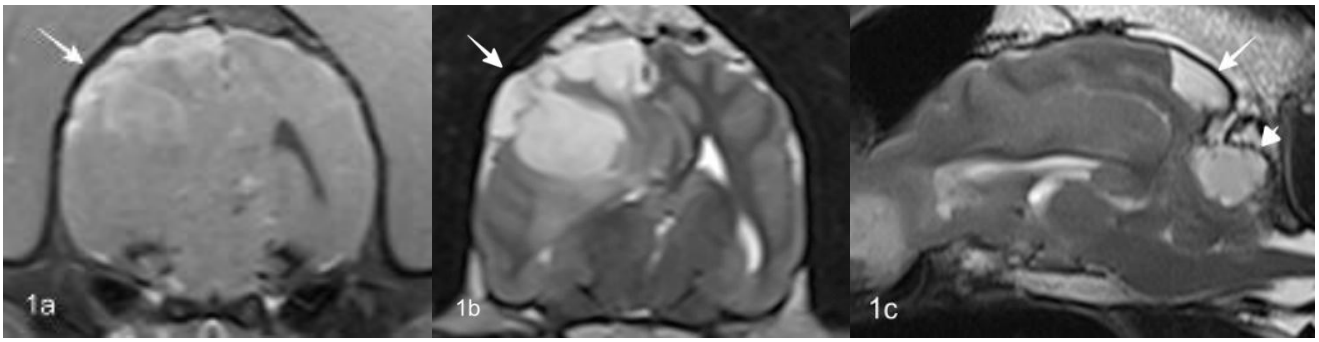

Figure 1 (a) Transverse T1-weighted MR image at the level of right cerebral hemisphere showing the hyperintense mass at the dorsal meninges (arrow) of the brain on post-contrast image. (b) Transverse T2-weighted MR image illustrating hyperintense masses at the right cerebral hemisphere (arrow) that resulted in compression of the right lateral ventricle and subsequent falcine herniation. (c) Saggital T2-weighted image demonstrating masses at the right occipital lobe (arrow) and left dorsal cerebellum (arrowhead).

As the dog did not show any improvement following a palliative treatment, right rostrotentorial craniectomy was performed to remove the mass completely at the right cerebral cortex. Coma score, vital signs, blood pressure and blood gas analysis were monitored every 2-4 hours. Ceftriaxone (10-20 mg/kg IV every 8 hours), metronidazole (15 mg/kg IV every 24 hours), morphine (0.5 mg/kg), phenobarbital (3.2 mg/kg orally every 12 hours) were administered postoperatively. In addition to the impression smears made during the surgery for cytological evaluation, the tumor sample was collected and fixed in 10% formalin for histopathological, histochemical and immunohistochemical evaluation. Postoperatively, the dog was maintained on anticonvulsant and corticosteroid therapy and survived for 6 months. Although the mass at the right cerebral cortex was removed during the first operation, seizures recurred, which led to the second operation. This time suboccipital craniectomy was performed to remove the

mass at the caudodorsal cerebellum with the objective to decrease the compression at pons and medulla oblongata. Nevertheless, the dog died during recovery period after a seizure for unforeseen reasons. The biopsy samples obtained were submitted for histopathological examination.

Materials and Method

During the operation impression smears were made from the mass and stained with Wright-Giemsa stain for cytological evaluation. Tissue samples of the brain mass obtained by surgical removal at the right cerebral cortex were processed for a conventional paraffin-embedding, and consequently sliced to 5 µm thick sections. These sections were stained with hematoxylin and eosin (HE) for histopathological evaluation and with periodic acid-Shiff (PAS) for glycogen in cytoplasmic granules and Oil-Red O for lipid. Moreover, these sections were used for immunohistochemical analysis for a number of proteins including Glia fibrillary acidic protein (GFAP), Neuron-specific enolase (NSE), Vimentin, Desmin, S-100 protein, CK14, CK8/18, CK19, and Ki67 using primary monoclonal antibodies (Table 1) to confirm the origin of tumor cells. GFAP is protein expressed by numerous cell types of the central nervous system including astrocytes and ependymal cells (Jacque et al., 1978). NSE was used to detect neuroendocrine cells or neoplasms. The tissue sections were processed according to the manufacturer's instructions (Leica Microsystems Bond maX System and Bond Polymer Refine Detection kit, Leica Microsystems, Bannockburn, IL, USA). Briefly, slides were incubated at 60°C for 60 minutes in Bond Dewax Solution. Epitope retrieval was performed by incubating the slides in Bond Epitope Retrieval Solution 2 at 100°C for 20 minutes. A 3-step indirect immunoperoxidase technique was followed. Primary antibody was applied for 45 minutes at room temperature followed by 3 consecutive rinses with Bond Wash Solution. Peroxide block (3% hydrogen peroxide) was then applied for 5 minutes and then rinsed 3 times with Bond Wash Solution. Post Primary Polymer was applied for 9 minutes before rinsing 3 times with Bond Wash Solution. Polymer Poly-HRP IgG was applied for 7 minutes and rinsed 3 times with Bond Wash Solution. The sections were washed once with deionized water before applying diaminobenzidine chromogen for 4 minutes, and then rinsed 3 times with deionized water. The slides were counterstained with hematoxylin for 5 minutes. Appropriate positive and negative control tissues used routinely in our laboratory were included in all staining procedures.

Table 1 List of proteins tested using antibodies. Dilution rates of the antibodies and their manufacturers' details are given. Results of immunohistochemical staining of sections of granular cell tumor in brain are shown in the last column.

Proteins tested	Dilution rate	Source	Results
Glia fibrillary acidic protein (GFAP)	1:2400	Dako	-
Neuron-specific enolase (NSE)	1:200	Cell Marque	-
Vimentin	1:800	Cell Marque	+
Desmin	1:200	Cell Marque	-
S-100 protein	1:1000	Cell Marque	-
CK14	1:200	Novocastra Laboratories	-
CK 8/18	1:600	Invitrogen	-
CK19	1:800	Cell Marque	-
Ki 67	1:200	Dako Corp	-

Results and Discussion

The cytological evaluation of impression smears showed high cellularity with both individual and clustered cells embedded in the pink matrix. These cells were large and round, and had abundant light pink cytoplasm that contained variable amounts of fine granular material dispersed throughout the cytoplasm (Figure 2a). Cytological findings from the masses obtained after the 1st surgery (right rostrotentorial craniectomy) and the 2nd surgery (suboccipital craniectomy) were similar and led towards the diagnosis of the granular cell tumors (GCTs).

Histopathological features revealed large, round to oval, neoplastic cells which were occasionally separated by thin fibrous connective tissue. Cytoplasm was light pink and slightly granular, whereas nuclei had dense chromatin and were eccentric (Figure 2b).

With PAS staining cytoplasmic granules of the neoplastic cells stained weakly (Figure 2c). The cytoplasmic vacuoles of neoplastic cells were unstained with Oil-Red O. The large granular cells showed scattered immunopositivity for vimentin localized in cytoplasm (Figure 2d), but showed immunonegativity for GFAP, NSE, Desmin, S-100, CK14, CK8/18, CK19, and Ki67. Taken together, these findings revealed that this intracranial tumor was a granular cell tumor which histologically resembled the intracranial GCTs reported previously in rats, a cat, and dogs (Higgins et al., 2001; Mandara et al., 2006; Spoor et al., 2013). Magnetic resonance (MR) imaging characteristics for GCTs are variable, although most

reports demonstrate some degree of contrast enhancement in MR images. The GCTs reported here appeared to be hypointense in the T1W image, which is different from previously reported hyperintensity in T1W images (Anwer et al., 2013) which is relatively uncommon in canine meningiomas (Higgins et al., 2001; Anwer et al., 2013). The T2W images revealed hyperintense masses as reported previously (Anwer et al., 2013). However, the high variation observed with this technique elucidates the difficulty of making a specific tumor diagnosis based on neuroimaging features alone.

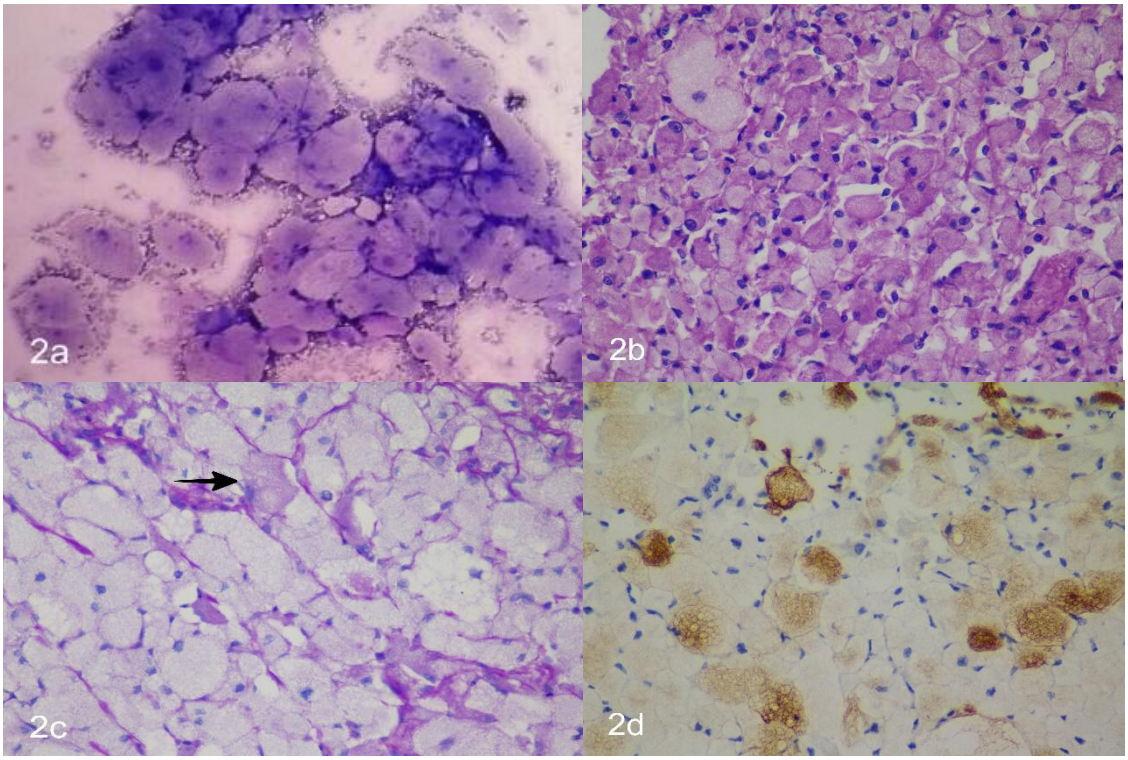

Figure 2 ((a) Impression smear of an intracranial cerebral mass stained with Wright-Giemsa stain showing high cellularity with individualized cells embedded in pink matrix; the round cells had medium blue cytoplasm containing pink granular material and numerous vacuoles. (b) Sections stained with HE showing neoplastic cells with eosinophilic cytoplasm containing fine granules; nuclei were small and eccentric. (c) Sections stained with PAS revealing mild positive staining of neoplastic cell (arrow). (d) Immunohistochemical analysis showing scattered neoplastic cells positive for vimentin.

The evidence based upon gross, histological and ultrastructural studies suggests that meningeal GCTs in rats could originate from meningeal arachnoid cells (Yoshida et al., 1997), however, some of these tumors may also contain mixtures of different cell types. In canine, cerebral GCTs appear to be of meningeal, mesenchymal, or neuronal origin (Higgins et al., 2001; Liu et al., 2004). On the other hand, in humans, most of the intracranial GCTs occur in the neurohypophysis and are thought to be derived from pituicytes while those reported in the skin, tongue, breast, and biliary and gastrointestinal systems are considered to be of Schwann cell origin (Carvalho et al., 1994). The negative staining for GFAP and cytokeratin expression observed in this study excluded the astroglial and choroid plexus origin of the reported tumor. Complete identity of the origin of the neoplastic cells in the present case could not be determined by immunohistochemistry, however, the strong binding of vimentin antibody supported a mesenchymal origin. Despite a number of reports, the origin of cells of GCTs in dogs is still controversial and not quite resolved, probably due to the fact that most of the times decision is based primarily on exclusionary rather than confirmatory data.

In conclusion, based on the cytological and histological observations, the present study demonstrated that the Golden Retriever with multiple intracranial granular cell tumors (GCTs) was able to undergo surgery and survive for 6 months. Therefore, the combination of surgical removal along with medical treatment can prolong the survival of a dog with GCTs. Although not conclusive, this study supports the notion that the granular cells may be of the meningeal origin.

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บทคัดย่อ

แนวทางการรักษาเนื้องอกในสมองชนิดแกรนูลาเซลล์ในสุนัขพันธุ์โกลเดน รีทรีฟเวอร์

พิชนันท์ ลีพหรัตนรักษ์[1*] ภัคธร ลิ่วเฉลิมวงศ์[1] มูฮัมหมัด คาลิด[3] กรรณิการ์ ศิริภัทรประวัติ[2]

สุนัขพันธุ์โกลเด้น รีทรีฟเวอร์ อายุ 9 ปี เพศเมีย ผ่านการทำหมัน มาด้วยอาการชัก ความรู้สึกตัวลดลง และเดินเซ จากการวินิจฉัยด้วยเครื่องสร้างภาพด้วยสนามแม่เหล็กไฟฟ้า (Magnetic Resonance Imaging) พบว่าสุนัขมีเนื้องอกในสมอง 5 ตำแหน่ง ได้แก่ ที่เยื่อหุ้มสมองซีกซ้าย ด้านบนของสมองเล็ก (เซรีเบลลัม) ผิวสมองใหญ่ซีกขวา (ซีรีบลัม) สมองส่วนออแฟกทอรี และสมองส่วนออกซิพิทอลซีกขวา สัตวแพทย์ตัดสินใจทำการผ่าตัดเปิดกะโหลกด้านขวาเพื่อเลาะเนื้องอกที่ผิวของสมองใหญ่ซีกขวาออก และส่งชิ้นเนื้อเพื่อวินิจฉัยทางจุลพยาธิวิทยา พบว่าก้อนเนื้อเป็นเนื้องอกแกรนูลาร์เซลล์ นอกจากนี้ ยังทำการตรวจด้วยวิธีอิมมูโนฮิสโตเคมี พบว่าก้อนเนื้องอกให้ผลย้อมสีเป็นบวกกับวิเมนติน และให้ผลย้อมสีเป็นลบกับ GFAP, NSE, Desmin, S-100, CK14, CK8/18, CK19 และ Ki67 หลังการผ่าตัด พบว่าความผิดปกติทางระบบประสาทดีขึ้น สุนัขมีอาการชักน้อยลง แต่ 6 เดือนหลังผ่าตัดสุนัขกลับมาแสดงอาการชักถี่มากขึ้นและมีอาการเดินเซมากขึ้น สัตวแพทย์จึงทำการผ่าตัดครั้งที่สอง โดยเลือกเปิดกะโหลกด้านท้ายออกเพื่อเลาะก้อนเนื้อที่ด้านบนของสมองเล็กออก และส่งชิ้นเนื้อเพื่อตรวจทางจุลพยาธิวิทยา พบว่าเนื้องอกเป็นเนื้องอกของแกรนูลาร์เซลล์เหมือนเนื้องอกก้อนแรก หลังการผ่าตัดสุนัขมีภาวะชักและเสียชีวิตในช่วงการพักฟื้นหลังผ่าตัด

คำสำคัญ: สุนัข สมอง การผ่าตัด เนื้องอกแกรนูลาร์เซลล์

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