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Case Report

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Abstract

Skin biopsied tissues from two dogs, initially diagnosed as hemophagocytic histiocytic sarcoma (HHS) by histopathology, were immunostained with a series of antibodies against lambda light chains, kappa light chains, multiple myeloma oncoprotein 1 (MUM-1), CD-3, paired box 5 (PAX-5), E-cadherin and lysozyme. Heteroduplex polymerase chain reaction for antigen receptor rearrangements (hPARR) was additionally performed on formalin-fixed paraffin-embedded (FFPE) tissues to rule out lymphomas from histiocytomas. Results showed that both cases were immunopositive for lambda light chains, MUM-1, PAK-5 and lysozyme, indicating a diagnosis of extramedullary plasmacytoma (EMP). Moreover, negative PCR result provided additional evidence confirming the diagnosis. Even though hPARR could not differentiate between histiocytomas and EMP, it could rule out these tumors as malignant lymphomas. This report emphasizes the advantage of using a panel of antibodies, besides histopathology, for HHS diagnosis.

Keywords: cutaneous extramedullary plasmacytoma, dog, immunophenotype

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Introduction

Canine extramedullary plasmacytoma (EMP), one of the common benign cutaneous round cell tumors, originates from plasma cells without the involvement of bone or bone marrow (Araújo et al., 2012; Gupta et al., 2014). Besides integuments, EMP has been infrequently reported in various organs including the gastrointestinal tract, third eyelid gland, uterus, scrotum, larynx, brain, esophagus, vulva, vertebral canal and lung (Jacobs et al., 2002; Hayes et al., 2007; Wright et al., 2008; Perlmann et al., 2009; Adelman et al., 2014). Recurrence and metastasis of the tumor were recorded. Multiple myeloma is significantly clinical relevant to dogs with EMP (Platz et al., 1999; Adelman et al., 2014). By routine hematoxylin and eosin (H&E) staining, the diagnosis of canine EMP is often confused with other round cell tumors such as cutaneous histiocytomas because they share similar histological and pleomorphic variations (Araújo et al., 2012). Both intracellular and extracellular eosinophilic proteinaceous substances can be produced by EMP, but likewise hemophagocytic histiocytic sarcomas (HHS), one of the variants of malignant histiocytomas, can engulf red blood cells and reveal eosinophilic intracytoplasmic substances. Therefore, immunohistochemistry (IHC) using a panel of antibodies is needed to characterize and differentiate these neoplasms for developing appropriate therapeutic plan and prognosis (Ramos-Vara et al., 2007).

Case History

Case 1: A 10-year-old male Siberian Husky that was presented to a private clinic with a chief complaint of a solitary subcutaneous mass of 0.5 x 1 cm located at the right forelimb digit.

Case 2: A 5-year-old male mongrel dog that was brought to the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University with a subcutaneous mass of 3 x 4 cm located at the perineum.

Diagnostic Investigations

Pathology: Both cases were initially evaluated by fine-needle aspirated (FNA) cytology and were both diagnosed with round cell tumors. Thereafter, excisional surgery was performed and tissues were submitted for routine histopathological processes at the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. Briefly, 4-µm thick formalin-fixed paraffin-embedded (FFPE) tissues were deparaffinized in xylene, rehydrated in serial decreasing concentrations of ethanol, stained with hematoxylin and eosin (H&E), dehydrated with increasing concentrations of ethanol, and permounted by coverslip slides. Microscopic findings were inspected under a light microscope.

Immunohistochemistry (IHC): In order to rule out and differentiate a series of round cell tumors, the 4-µm thick FFPE sections from both dogs (cases) were immunostained using antibodies against lambda light chains, kappa light chains, multiple myeloma 1 (MUM-1), CD-3, paired box 5 (PAX-5), E-cadherin and lysozyme. All IHC procedures were kindly performed by the Joint Pathology Center, Joint Task Force National Capital Region Medical Center, Maryland, USA.

Heteroduplex polymerase chain reaction for antigen receptor rearrangements (hPARR): Genomic DNA from 100-µm thick FFPE sections from these two cases, and other four skin tumors that included benign and malignant histiocytomas, B- and T-cell lymphomas, were extracted with a FFPE DNA purification kit (Norgen, Canada) following the manufacturer's instructions. Four sets of primer were used to identify the clonal expansion of B or T lymphocytes. The specific bands were composed of Cµ gene at 130 bp (Burnett et al., 2003), immunoglobulin heavy chain (IgH) gene at 80-150 bp (Tamura et al., 2006), and T-cell receptor gamma (TCRγ) gene at 55-90 bp (Yagihara et al., 2007). The PCR protocol and heteroduplex analysis were performed as previously reported (Tamura et al., 2006, Yagihara et al., 2007).

Results and Discussion

Morphologically, both masses were round to ovoid in shape, reddish-brown on the surface and of a firm consistency when palpated. Microscopically, the masses revealed round to ovoid pleomorphic cells arranged in sheet, nest and packet patterns. These cells had abundant eosinophilic cytoplasms, which contained one or more bright eosinophilic globular cytoplasmic inclusions. The mitotic figure count was high (8-9 per 400x high power field of view). Nuclei of both cases were round and centrally located, with finely to coarsely stippled chromatin. However, multinucleated and karyomegalic cells were frequently observed in case 1 than case 2, thereby this feature could be classified as "polymorphous blastic type" in case 1 (Fig. 1a) and "asynchronous type" in case 2 (Fig. 1b). The initial diagnosis went to canine hemophagocytic histiocytic sarcoma (HHS); however, canine cutaneous extramedullary plasmacytoma (EMP) could not be ruled out. Therefore, the IHC analysis was performed.

The IHC analysis revealed that most of the neoplastic cells were positive for lambda light chains, PAX-5, MUM-1 and lysozyme (Fig. 1c–f), but negative for kappa light chains, E-cadherin and CD-3. The immunophenotypes from these two dogs were consistent with a plasma cell tumor or plasmacytoma. Additionally, the eosinophilic cytoplasmic globules in the cytoplasm of some neoplastic cells stained intensely for lambda light chains; interpreted as Russell bodies and not examples of erythrophagocytosis. The results of the hPARR analysis revealed that these two cases including benign and malignant histiocytomas were negative for both the IgH and TCRγ genes.

Canine extramedullary plasmacytoma (EMP), a plasma cell proliferating disease, is a common skin tumor found on various parts of the body (Platz et al., 1999; Araújo et al., 2012). In this study, both tumors were determined as polymorphous blastic and

asynchronous types for the digit and perineal masses, respectively. The pleomorphic activity of EMP tumors often leads to their ambiguous diagnosis by routine histopathology. Previous studies have reported that histologically diagnosed mast cell tumors and cutaneous histocytomas were tentatively defined as cutaneous plasmacytoma by IHC analysis in 57.1% and 42.9%, respectively, of cases (Araújo et al., 2012). Moreover, EMPs can produce immunoglobulin that is often seen as an eosinophilic inclusion in the cytoplasm of the tumor cells, which could be confused with erythrocyte-engulfed HHS. Therefore, the application of IHC using a panel of specific antibodies has been used to differentiate the cell origin of such tumors.

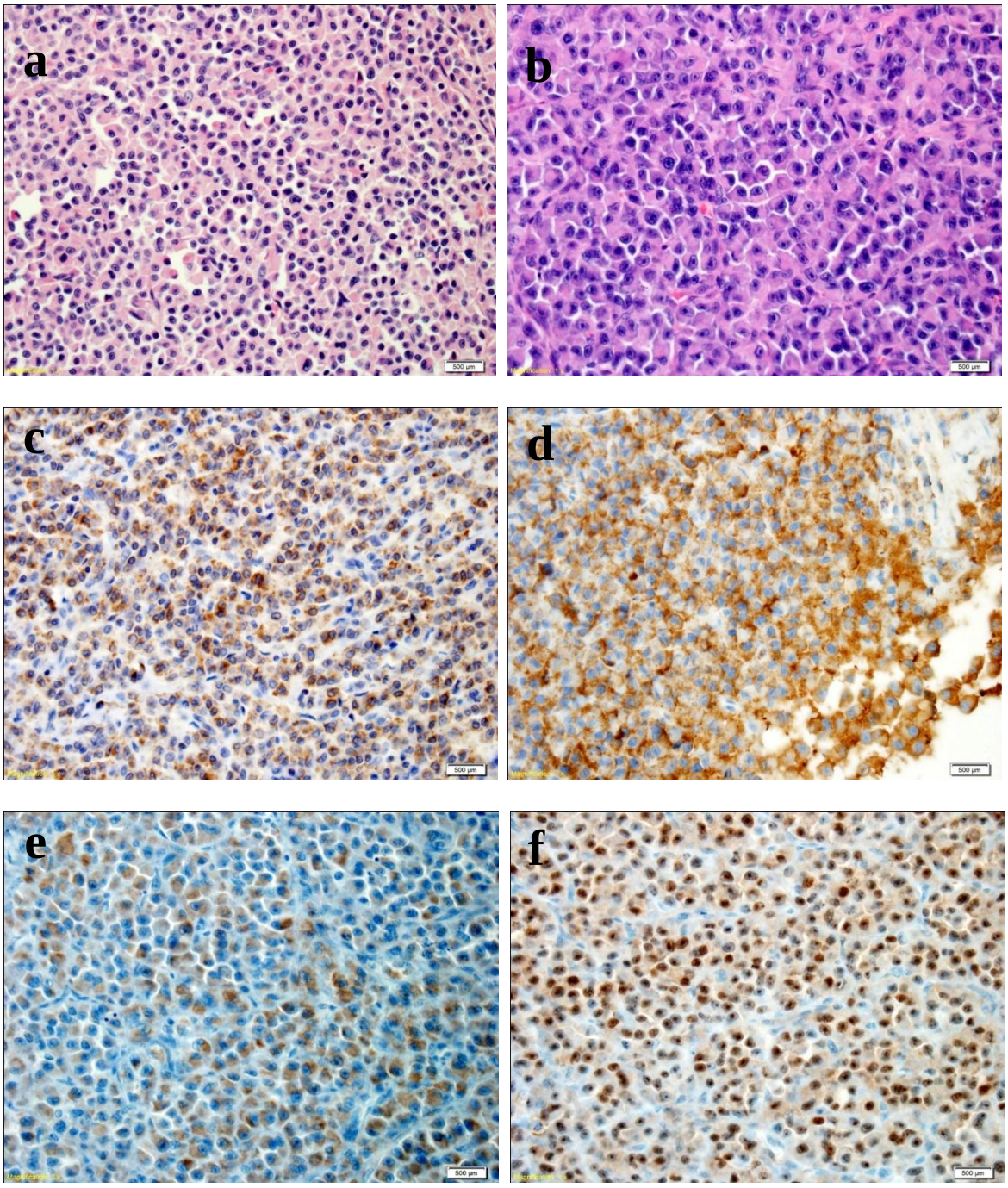

Figure 1 Canine extramedullary plasmacytoma (EMP). (a) Case 1, right forelimb digit mass, polymorphous blastic type of EMP and H&E stain. (b) Case 2, perineal mass, asynchronous type of EMP and H&E stain. (c) Case 1, (d) Case 2, lambda light chains antibody and IHC, by the ABC method. (e) Case 2, PAK-5 antibody and IHC by the ABC method. (f) Case 2, MUM-1 antibody and IHC by the ABC method. Scale bar = 500 µm.

In this report, CD3 and PAX-5 were used for the detection of T-cell and B-cell lymphocytes, respectively (Willmann et al., 2009; Sirivisoot et al., 2016). In both dog cases the samples were immunonegative for CD3, and so the tumor origin was not T lymphocytes. The tumors were both E-cadherin negative, suggesting that they might not originate from Langerhans cells. However, they were positively stained with lysozyme, which rules out human plasmacytoma cells (Bayer-Garner et al., 2003). The

tumor cells showed immunoreactivity with lambda light chains, especially in their cytoplasmic globules, resembling Russell bodies. These findings indicated the absence of any erythrophagocytic activity of HHS. Both cases were negative for kappa light chains, which can possibly be immunopositive in EMP. Nonetheless, some recent reports have argued that kappa light chains are not stained in 20% of EMP cases (Shameem et al., 2011). Accordingly, both cases were strongly immunolabeled with MUM-1 antibodies, which are rather specific and indicate a plasma cell origin, rather than staining with CD79a, the B-cell lymphocyte marker (Ramos-Vara et al., 2007). In addition, the hPARR analysis of the EMP samples presented negative results, similar to that of the histiocytomas. Thus, it was only confirmed that the tumor cells in these two cases were not derived from malignant T or B lymphomas, another round cell tumor with similar characteristic appearance.

In conclusion, the diagnoses of both cases in this study were canine EMP according to the results of the IHC analysis. By only histomorphological characterization, it was rather difficult to differentiate EMP from HHS because they share some similar findings, particularly the eosinophilic cytoplasmic inclusion bodies. Even though hPARR could rule out malignant lymphomas from EMP, it could not differentiate EMP from histiocytomas. Thus, the IHC assay outweighs other techniques for determining the origin of tumor. This study provides series of essential antibodies for IHC analysis for an effective diagnosis of plasma cell-originated tumors from other round cell cutaneous tumors. Moreover, it raises the awareness of the equivocal diagnosis of HHS based only on routine histology, which could result in inappropriate treatment plan and prognosis.

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บทคัดย่อ

ลักษณะปรากฏทางอิมมูนของเนื้องอกผิวหนังชนิดพลาสมาเซลล์ที่พบนอกไขกระดูกในสุนัขสองตัว

ฉัตรชัย ผิวบาง สิรินทรา ศิริวิสูตร สิทธิโชค ลาชโรจน์ อนุเทพ รังสีพิพัฒน์ สมพร เตชะงามสุวรรณ*

เนื้องอกผิวหนังจากสุนัข 2 ตัวได้รับการวินิจฉัยเบื้องต้นทางจุลพยาธิวิทยาว่าเป็น hemophagocytic histiocytic sarcoma (HHS) ต่อมาทำการทดสอบเพิ่มเติมด้วยการย้อมด้วยแอนติบอดีที่จำเพาะต่อ lambda light chains, kappa light chains, multiple myeloma oncoprotein 1 (MUM-1), CD-3, paired box 5 (PAX-5), E-cadherin และ lysozyme ทำการทดสอบแยกแยะเนื้องอกชนิด lymphoma ออกจาก histiocytoma ด้วยเทคนิคทางอณูชีววิทยา hPARR บนชิ้นเนื้อพาราฟิน ผลการตรวจพบว่า เนื้องอกผิวหนังสุนัขทั้ง 2 ตัวอย่างให้ผลบวกทางอิมมูนกับแอนติบอดี lambda light chains, MUM-1, PAK-5 และ lysozyme ซึ่งบ่งชี้การวินิจฉัยว่าเป็นเนื้องอกผิวหนังชนิดพลาสมาเซลล์ที่พบนอกไขกระดูก (extramedullary plasmacytoma; EMP) นอกจากนี้ การตรวจด้วยเทคนิค hPARR ยังให้ผลลบ ถึงแม้ว่าจะไม่สามารถวินิจฉัยแยกแยะระหว่างเนื้องอกชนิด histiocytoma กับ EMP ได้ แต่เทคนิคนี้สามารถแยกได้ว่าเนื้องอกเหล่านี้ไม่ได้เป็นเนื้องอกชนิด lymphomas รายงานกรณีศึกษาในครั้งนี้มุ่งเน้นให้เห็นถึงความสำคัญของการวินิจฉัยเพิ่มเติมโดยใช้แอนตีบอดีที่จำเพาะ นอกเหนือจากเทคนิคทางจุลพยาธิวิทยาในการวินิจฉัย HHS

คำสำคัญ: เนื้องอกผิวหนังชนิดพลาสมาเซลล์ที่พบนอกไขกระดูก สุนัข ลักษณะปรากฏทางอิมมูน

หน่วยปฏิบัติการวิจัยโรคมะเร็งในสัตว์เลี้ยง ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330
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