doi: 10.17221/7987-VETMED

Cutaneous extrarenal rhabdoid tumor in a dog: a case report

H.J. Kim[1], E.J. Choi[1], H.R. Lee[1], B.T. Kwon[2], S.H. Do[1]

[1]College of Veterinary Medicine, Konkuk University, Seoul, Republic of Korea
[2]Taejong Animal Hospital, Busan, Republic of Korea

ABSTRACT: Rhabdoid tumours (RTs) are rare, highly aggressive tumours of undetermined origin in humans, and are sub-classified as renal/extrarenal RTs depending on location. The origins of extrarenal rhabdoid tumours are an enigma and neoplasms have rarely been reported in non-primate species. An 11-year-old male Maltese dog was presented with a submandibular mass. Histologically, the mass was composed of sheets of highly pleomorphic "rhabdoid" cells, further characterised by the presence of large epithelioid cells with globular/fibrillar paranuclear inclusions. Further immunohistochemical analysis revealed that the neoplastic cells were positive for vimentin and desmin similar to human tumours. In addition, ultrastructural analysis showed that the intracytoplasmic inclusions were mainly composed of whorled bundles of intermediate filaments. Our results suggest a useful diagnostic approach to cutaneous, extrarenal rhabdoid tumours in dogs and describe their characteristics.

Keywords: rhabdoid tumour; cutaneous; cytoplasmic inclusion; vimentin; transmission electron microscopy

Rhabdoid tumours (RTs) are rare, highly aggressive tumours occurring mainly in the kidneys of human infants or children (Weeks et al. 1989). Extrarenal RTs may occur in sites such as the brain, soft tissues, uterus, bladder, prostate gland, liver, thymus, skin and orbit as reported in humans (Biggs et al. 1987; Wick et al. 1995; Rorke et al. 1996; Oda and Tsuneyoshi 2006). Due to their highly aggressive clinical behaviour, extrarenal RTs exhibit frequent recurrence and are characterised by distant metastasis leading finally to poor prognosis (Tsuneyoshi et al. 1985; Fuijoka et al. 2013).

Histologically, RTs are characterised by sheets of highly pleomorphic "rhabdoid" cells which resemble rhabdomyosarcoma under the light microscope and which rely on the presence of large epithelioid cells with eccentric eosinophilic cytoplasm, vesicular nuclei, and prominent nucleoli (Petitt et al. 2005; Izawa et al. 2008). In contrast to rhabdomyosarcoma, RT cells often possess eosinophilic, globular/fibrillar paranuclear inclusions and show various amounts of reactivity to lineage specific-immunohistochemical markers such as those targeted at mesenchymal cells, smooth muscle, skeletal muscle, melanocytes, epithelial cells and nerves (Fanburg-Smith et al. 1998; Morgan et al. 2000).

In animals, apart from experimental RT modelling in mice, fewer than 10 cases of renal and/or extrarenal RT have been reported in the literature. Most cases of RT in veterinary medicine exhibited similar histopathological features to those of human RT. However, in contrast to human cases, most of these RT cases occurred in adults and extrarenal RTs, such as those of the gastric wall (Schauer et al. 1994), brain (Steele et al. 1997), orbit (Hong et al. 1999) and skin (Izawa et al. 2008), were more frequently reported than renal RTs.

Primary presentation in the skin is exceedingly rare and to the best of our knowledge, only two cases of extrarenal RT were reported in a dog (Chung and Do 2009) and a cat (Izawa et al. 2008). Furthermore, prognosis differs markedly in cutaneous RTs compared to extrarenal RTs from other locations. Most extrarenal RTs are fatal but, extrarenal RT of the cutaneous/subcutaneous region is localised in specific regions without metastasis.

doi: 10.17221/7987-VETMED

In this article, a case of a cutaneous, extrarenal RT is reported and the immunohistochemical and ultrastructural features are discussed with regard to the exact diagnosis of extrarenal RT according to the present classification.

Case description

An 11-year-old male Maltese dog developed a small cutaneous nodule in the submental area. Grossly, the white-coloured mass was 2 × 2 × 1 cm in size and firm on palpation. Dark-to-yellow, mucoid fluid was oozing from the section. After surgery, no recurrence of the tumour was apparent at the surgical site at follow-ups for more than one year.

For histological and histochemical analysis, the specimen was fixed in 10% neutral-buffered formalin, embedded in paraffin, and sections of 4 µm thickness were cut. These sections were stained with haematoxylin and eosin (H&E), Masson's trichrome stain and Periodic Acid-Schiff stain for differential diagnosis from rhabdomyosarcoma. For immunohistochemistry, the following primary antibodies were used: α-SMA (Sigma-Aldrich, St. Louis, USA, 1 : 100), CD45 (Santa Cruz Biotechnology, CA, USA, 1 : 100), CD68 (Dakocytomation, Glostrup, Denmark, 1 : 100), desmin (Dakocytomation, Glostrup, Denmark, 1 : 100), GFAP (Dakocytomation, Glostrup, Denmark, 1 : 1000), HMB45 (Dakocytomation, Glostrup, Denmark, 1 : 100), MyoD1 (Sigma-Aldrich, St. Louis, USA, 1 : 100), pancytokeratin (Abcam, Cambridge, UK, 1 : 100), S100 (Abcam, Cambridge, UK, 1 : 100), vimentin (Dakocytomation, Glostrup, Denmark, 1 : 100). The antibody-labelled sections were then incubated with an avidinbiotin-peroxidase complex (ABC) solution using an ABC kit (Invitrogen, Carlsbad, CA, USA). 3,3'-Diaminobenzidine (Zymed Laboratories, CA, USA) was used for visualisation. The sections were counterstained with Mayer's haematoxylin.

For transmission electron microscopy (TEM), four excisional biopsy samples of the skin were fixed in 6.25% glutaraldehyde in phosphate buffered saline (PBS) at 8 °C for 48 h, post-fixed in 1% osmium tetroxide, and dehydrated and embedded in Epon according to standard procedures. Ultra-thin sections were cut with an Ultracut E microtome and stained with lead citrate and uranyl acetate. TEM was performed using a Zeiss EM 10 electron microscope (Carl Zeiss, Jena, Germany). Images were photographed at × 8000 magnification in a standard manner.

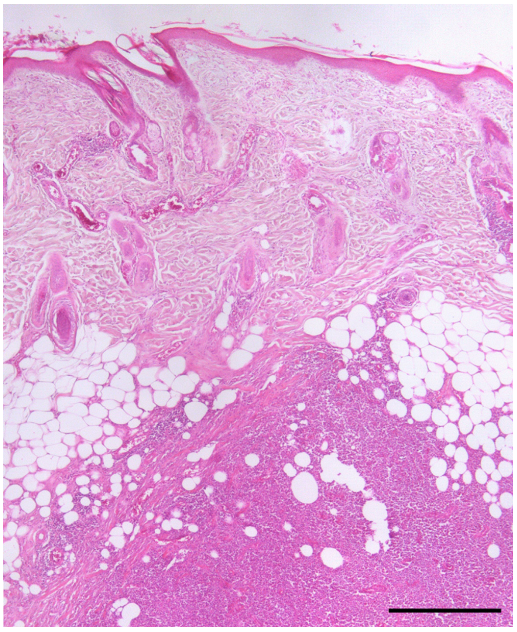

Figure 1. Histopathological findings in the submandibular region. Non-cohesive sheets or a nest of round-to-polygonal cells infiltrated from the dermis to subcutaneous adipose tissues (haematoxylin-eosin, bar = 500 µm)

Microscopic examination of the skin revealed a high cellularity of tumour cells composed of non-cohesive sheets or a nest of round-to-polygonal cells. Most of the tumour cells had hyperchromatic oval-to-round nuclei with abundant eosinophillic cytoplasm in the subcutis. Neoplastic cells had infiltrated to the dermis and showed further extension into the subcutaneous adipose tissue and

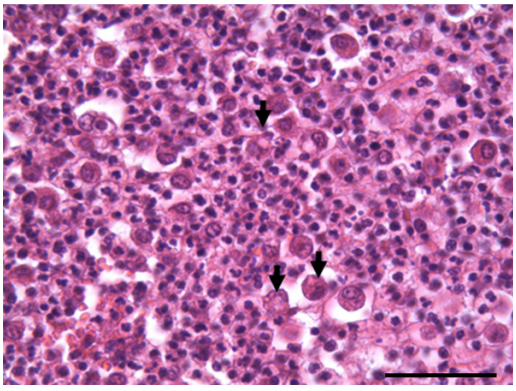

Figure 2. Two distinctive cellular phenotypes in the neoplastic lesion. Neoplastic cells consisted of poorly differentiated epithelioid cells and 'rhabdoid cells' which had paranuclear, globular, hyaline, eosinophilic, and cytoplasmic inclusion bodies. (haematoxylin-eosin, bar = 50 µm)

doi: 10.17221/7987-VETMED

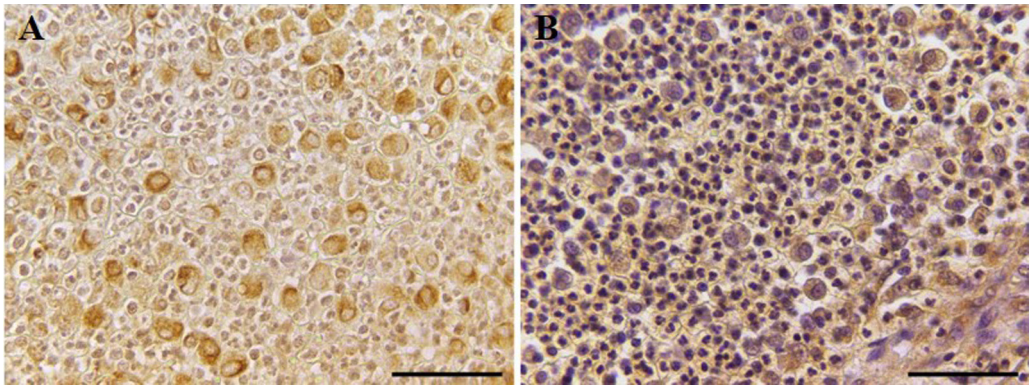

Figure 3. Immunohistological characterisation of rhabdoid cells. Immunohistochemistry revealed a strong positive reaction for vimentin in the cytoplasm of rhabdoid cells (**A**). The tumour cells also showed a positive immunoreaction for desmin (**B**). (A–B = immunohistochemistry, bars = 50 μm)

muscularis (Figure 1). Necrotic foci were scattered throughout the neoplasm, along with mixed cellularity of inflammatory cells such as lymphocytes, plasma cells, neutrophils, and macrophages. In focal areas, the tumour cells were intermixed with embracing cells, which had pale nuclei and clear cytoplasm showing a 'spongy' appearance.

Throughout the neoplastic lesion, the neoplastic cells exhibited two distinctive phenotypes. One phenotype consisted of poorly differentiated epithelioid cells with scant cytoplasm and coarse chromatin with variable prominent nucleoli (Figure 2). The other phenotype showed 'rhabdoid' features and was characterised by paranuclear, globular, hyaline, eosinophilic, and cytoplasmic inclusion bodies (arrow of Figure 2). The latter cells were larger than the former ones with abundant eosinophilic cytoplasm, eccentrically located round-to-ovoid nuclei, vesicular chromatin, and prominent nucleoli.

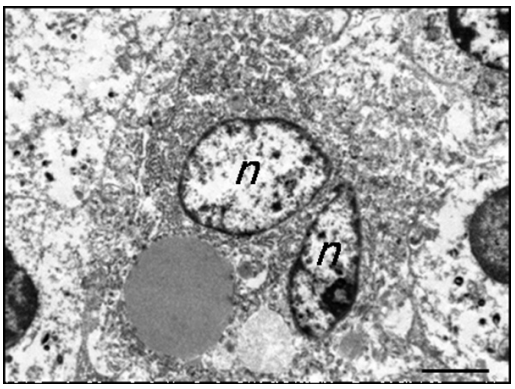

Figure 4. Ultrastructural findings of rhabdoid cells. Most tumour cells contained eccentric nuclei with oval-shaped intracytoplasmic inclusion bodies and vaguely interfilamentous material (n = nuclei, bar = 2 μm)

At higher magnification, rhaboid cells were revealed to be irregular in shape. They stained pink by H&E stain and blue by Masson's trichrome stain, but did not react with Periodic Acid Schiff stain (data not shown). Immunohistochemically, the neoplastic cells were positive for vimentin (Figure 3A) and desmin (Figure 3B). However, they did not react with antibodies against α-SMA, CD45, CD68, GFAP, HMB45, MyoD1, pancytokeratin and S100 (data not shown).

Electron microscopy revealed that neoplastic cells possessed intermediate filaments with small amounts of entrapped cytoplasmic organelles. They also contained small, oval-shaped intracytoplasmic inclusion bodies, which were composed of densely packed granulovesicular and vaguely filamentous material (Figure 4).

DISCUSSION AND CONCLUSIONS

We here report the morphological, immunohistochemical and ultrastructural features of a cutaneous, extrarenal RT. RT was originally described as a "rhabdomyosarcomatous variant" of Wilms' tumour in the kidney (Beckwith and Palmer 1978). However, despite its morphological similarity under the light microscope, it soon became apparent that the immunohistochemical, ultrastructural and clinicopathological features differed between rhabdoid and Wilms' tumours (Haas et al. 1981; Weeks et al. 1989). According to previous reports (Izawa et al. 2008), both renal and extrarenal RTs showed a similar histomorphological appearance including nests or non-cohesive sheets of round to polygonal cells with pale circular regions outlined by a eo-

doi: 10.17221/7987-VETMED

sinophilic rim of cytoplasm or dense eosinophilic inclusions. The peculiar and "pathognomonic" feature of the RT cells is their eccentric nuclei with the presence of intracytoplasmic inclusions.

The histological findings of RT in skin were described to be very similar, but the immunochemical reactivity was variable, with the exception of a strong positive immunoreaction for vimentin (Fanburg-Smith et al. 1998; Petitt et al. 2005; Izawa et al. 2008). Consideration of another case prompted the suggestion that epithelioid cell morphology along with positive immunohistochemical staining for vimentin, desmin, CD56, CD10, and WT-1 support an underlying poorly differentiated rhabdomyosarcoma (Petitt et al. 2005). However, another study reported that immunopositivity for vimentin, neuron-specific enolase, neurofilament, and S-100 protein may suggest a neuroectodermal origin for the tumour (Izawa et al. 2008). In the present study, pronounced immunoreactions were noted for vimentin and desmin, but not for other specific markers such as for the neuronal lineage (GFAP, S100), myogenic lineage (α-SMA, MyoD1), epithelial lineage (pancytokeratin), inflammatory cells (CD45, CD68) and melanocytes (HMB45). Thus, the various immunoreactions observed in the present case have failed to clarify the cellular origin of RTs. It has been postulated that a neoplasm with features of RT that develops in different tissues may be distinguished by its histogenetic origin, while sharing common morphological and immunocytochemical phenotypes (Kuroda et al. 2005). In this regard, ultrastructural features that are suggestive of a rhabdoid cell include a filamentous cytoplasm and paranuclear inclusions.

Human RT is rare and is found mostly in neonates or children; it shows a very aggressive clinical behaviour including reoccurrence and distant metastasis. Extrarenal RT has also been reported as a primary neoplasm in the brain, skin, liver, thymus, and orbit. In contrast to humans, most RTs in adult animals are extrarenal. Previously reported non-cutaneous, extrarenal RTs in orangutan, dog, and horse cases metastasised to adjacent organs and lymph nodes. However, similar to the present case, cutaneous RTs of a cat displayed no recurrence and no metastasis after surgical excision (Izawa et al. 2008). Thus, the incidence of extrarenal RTs in humans and dogs differs especially with regard to the occurrence of cutaneous/subcutaneous extrarenal RTs in adults.

Recent cytogenetic studies of RTs in humans have revealed that the SMARCB1/hSNF5/INI1 tumour suppressor gene located on 22q11 is deleted or mutated in the central nervous system, and in renal and extrarenal regions (Versteege et al. 1998; Biegel et al. 2002). A recent RT-PCR-based study demonstrated that the hSNF5/INI1 gene, a suppressor gene of malignant RT that maps to 22q11.2, is homozygously deleted from exons 1–5 in RT cell lines (Kuroda et al. 2005). Furthermore, the expression of two other tumour suppressor genes, p16 and p53, was not detected by RT-PCR. This raises the possibility that the aggressive phenotype of malignant RTs is caused by the loss of two or more tumour suppressor genes (Kuroda et al. 2005). Therefore, further molecular genetic analysis is needed to demonstrate the relationship between the mutation of the hSNF5/INI1/SMARCG1 locus on 22q11.2 and the occurrence of extrarenal RT in animals.

In conclusion, our case reveals similarities between the cutaneous, extrarenal RT of a Maltese dog and RTs characterised in previous studies by immunochemical profiles (Morgan et al. 2000; Petitt et al. 2005). Additionally, electron microscopy examinations of the tumour cells would be helpful in the diagnosis of RT. However, to perform comparative diagnosis of RT, further studies are needed, especially those combining molecular diagnosis with histological and immunochemical analysis.

REFERENCES

Beckwith JB, Palmer NF (1978): Histopathology and prognosis of Wilms tumors: results from the First National Wilms' Tumor Study. Cancer 41, 1937–1948.

Biegel JA, Tan L, Zhang F, Wainwright L, Russo P, Rorke LB (2002): Alterations of the hSNF5/INI1 gene in central nervous system atypical teratoid/rhabdoid tumors and renal and extrarenal rhabdoid tumors. Clinical Cancer Research 8, 3461–3467.

Biggs PJ, Garen PD, Powers JM, Garvin AJ (1987): Malignant rhabdoid tumor of the central nervous system. Human Pathology 18, 332–337.

Chung JI, Do SH (2009): Rhabdoid tumor in the gluteal region of a Shih-tzu dog. Korean Journal of Veterinary Research 49, 361–363.

Fanburg-Smith JC, Hengge M, Hengge UR, Smith JS Jr, Miettinen M (1998): Extrarenal rhabdoid tumors of soft tissue: a clinicopathologic and immunohistochemical study of 18 cases. Annals of Diagnostic Pathology 2, 351–362.

doi: 10.17221/7987-VETMED

Fujioka M, Hayashida K, Murakami C, Hisaoka M, Oda Y, Ito M (2013): Cutaneous malignant rhabdoid tumor in the palm of an adult. Rare Tumors 5, e36.

Haas JE, Palmer NF, Weinberg AG, Beckwith JB (1981): Ultrastructure of malignant rhabdoid tumor of the kidney. A distinctive renal tumor of children. Human Pathology 12, 646–657.

Hong CB, Van Meter PW, Latimer CL (1999): Malignant rhabdoid tumour in the orbit of a horse. Journal of Comparative Pathology 121, 197–201.

Izawa T, Yamate J, Takeda S, Kumagai D, Kuwamura M (2008): Cutaneous rhabdoid tumor in a cat. Veterinary Pathology 45, 897–900.

Kuroda H, Moritake H, Sawada K, Kuwahara Y, Imoto I, Inazawa J, Sugimoto T (2005): Establishment of a cell line from a malignant rhabdoid tumor of the liver lacking the function of two tumor suppressor genes, hSNF5/INI1 end p16. Cancer Genetics and Cytogenetics 158, 172–179.

Morgan MB, Stevens L, Patterson J, Tannenbaum M (2000): Cutaneous epithelioid malignant nerve sheath tumor with rhabdoid features: a histologic, immunohistochemical, and ultrastructural study of three cases. Journal of Cutaneous Pathology 27, 529–534.

Oda Y, Tsuneyoshi M (2006): Extrarenal rhabdoid tumors of soft tissue: clinicopathological and molecular genetic review and distinction from other soft-tissue sarcomas with rhabdoid features. Pathology International 56, 287–295.

Petitt M, Doeden K, Harris A, Bocklage T (2005): Cutaneous extrarenal rhabdoid tumor with myogenic differentiation. Journal of Cutaneous Pathology 32, 690–695.

Rorke LB, Packer RJ, Biegel JA (1996): Central nervous system atypical teratoid/rhabdoid tumors of infancy and childhood: definition of an entity. Journal of Neurosurgery 85, 56–65.

Schauer G, Moll R, Walter JH., Rumpelt HJ, Goltenboth R (1994): Malignant rhabdoid tumor in the gastric wall of an aged orangutan (Pongo pygmaeus). Veterinary Pathology 31, 510–517.

Steele KE, Schulman FY, Mena H, Strimple EO (1997): Rhabdoid tumor in the brain of a dog. Veterinary Pathology 34, 359–363.

Tsuneyoshi M, Daimaru Y, Hashimoto H, Enjoji M (1985): Malignant soft tissue neoplasm with histologic features of renal rhabdoid tumors: an ultrastrucural and immunohistochemical study. Human Pathology 16, 1235–1242.

Versteege L, Sevenet N, Lange J, Rousseau-Merck MF, Ambros P, Handgretinger R, Aurias A, Delattre O (1998): Truncating mutation of hSNF5/INI1 in aggressive paediatric cancer. Nature 394, 203–206.

Weeks DA, Beckwith JB, Mierau GW, Luckey DW (1989): Rhabdoid tumor of kidney. A report of 111 cased from National Wilms' Tumor Study Pathology Center. American Journal of Surgical Pathology 13, 439–458.

Wick MR, Ritter JH, Dehner LP (1995): Malignant rhabdoid tumors: a clinicopathologic review and conceptual discussion. Seminars in Diagnostic Pathology 12, 233–248.

Received: 2014–09–26
Accepted after corrections: 2015–01–23

Corresponding Author:

Sun Hee Do, Konkuk University, College of Veterinary Medicine, Seoul 143-701, Republic of Korea
E-mail: shdo@konkuk.ac.kr