Case Report

Subendocardial nodular proliferation of myofibroblasts in a laboratory beagle

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Abstract: A smooth white focus was macroscopically observed in the right ventricular endocardium in a 15-month-old male beagle from a 4-week oral gavage toxicity study. This lesion likely arose from myofibroblasts and was diagnosed as subendocardial nodular proliferation of myofibroblasts. This lesion was observed only in one animal in a low dose group and was an incidental finding. Histopathologically, the well-demarcated nodule comprised abundant collagen containing spindle cells arranged in intermediate to long streams and formed broad interlacing fascicles. The spindle cells had an indistinct cell border with round to elongated hyperchromatic nuclei or nuclei with finely stippled chromatin and indistinct nucleoli. Furthermore, these cells were weakly positive for S100 and positive for α-smooth muscle actin, calponin, and vimentin. Based on the histological features, the proliferating spindle cells resembled phenotypes of smooth muscles or myofibroblasts. However, the proliferating cells lacked well-differentiated smooth muscle cells, cigar-shaped nuclei, and well-developed reticulin fibers outlining individual cells. This study describes the morphological characteristics of an endocardial proliferative lesion in the right ventricle of a beagle. (DOI: 10.1293/tox.2019-0053; J Toxicol Pathol 2020; 33: 25–28)

Key words: subendocardium, α-smooth muscle actin (α-SMA)-positive, spindle cell, dog

Tumor incidence (0.19%) and proliferative cardiac lesions are uncommon in dogs[1]. Subendocardial/endocardial proliferative lesions are generally rare in laboratory animals. Rats are known to spontaneously develop endocardial proliferative lesions in long-term toxicity or carcinogenicity studies[2]. However, there is only one endocardial malignant peripheral nerve sheath tumor that was reported in an 8-year-old dog[3]. In this case study, we have investigated the histological features of an endocardial proliferative lesion in the right ventricle of a male beagle.

The male beagle (Kitayama Labes Co., Ltd., Yamaguchi, Japan) was part of the low dose group of a 4-week oral gavage toxicity study. No clinical signs were observed during this period. The experimental procedures were approved by the Institutional Animal Care and Use Committee of Shonan Research Center, Takeda Pharmaceutical Company Limited. The animal was euthanized at 15 months of age by exsanguination under anesthesia (with thiopental sodium) and subjected to complete necropsy. At necropsy, a smooth white focus was observed in the right ventricular endocardium (Fig. 1A). The tendinous cord was attached to the center of this focus. There were no macroscopic findings in the other organs. This lesion was observed only in a male dog in a low dose group and was considered as an incidental finding. The focus-containing heart was fixed in 10% volume by volume (v/v) neutral buffered formalin, embedded in paraffin, and sectioned; these sections were subjected to hematoxylin and eosin (H&E), Masson's trichrome (MT), Elastica van Gieson (EVG), and silver staining. We used immunohistochemistry to characterize the lesion using anti-cow S100 (diluted 1:500; Abcam, Cambridge, UK, ab52642), anti-human Schwann cell/peripheral myelin (Schwann/2E, diluted 1:2,500, Cosmo Bio, Tokyo, Japan, GU01-M01AS-A), anti-cow glial fibrillary acidic protein (GFAP, diluted 1:1, DAKO, Kyoto, Japan, IR524), anti-human α-smooth muscle actin (α-SMA, diluted 1:1,000; DAKO, BM0851), anti-human calponin (calponin, diluted 1:20; Abcam, ab46794), anti-human vimentin (diluted 1:500; Santa Cruz, San Diego, CA, USA, sc-6260), and anti-rat proliferating nuclear antigen (PCNA, 1:5,000; DAKO, M0879) antibodies.

Histopathologically, we observed a well-demarcated nodule that was moderately cellular, encapsulated, and expanded on the right ventricular endocardial surface (Fig. 1B). The nodule consisted of spindle cells arranged in intermediate to long streams that formed broad interlacing fascicles. These cells had indistinct cell borders and round to ovoid to elongated hyperchromatic nuclei with finely stippled chromatin and indistinct nucleoli. The spindle cells exhibited minimal anisocytosis and anisokaryosis and mitoses were rare (Fig. 1C and D). The presence of abundant collagen in the nodule was confirmed using MT and EVG staining
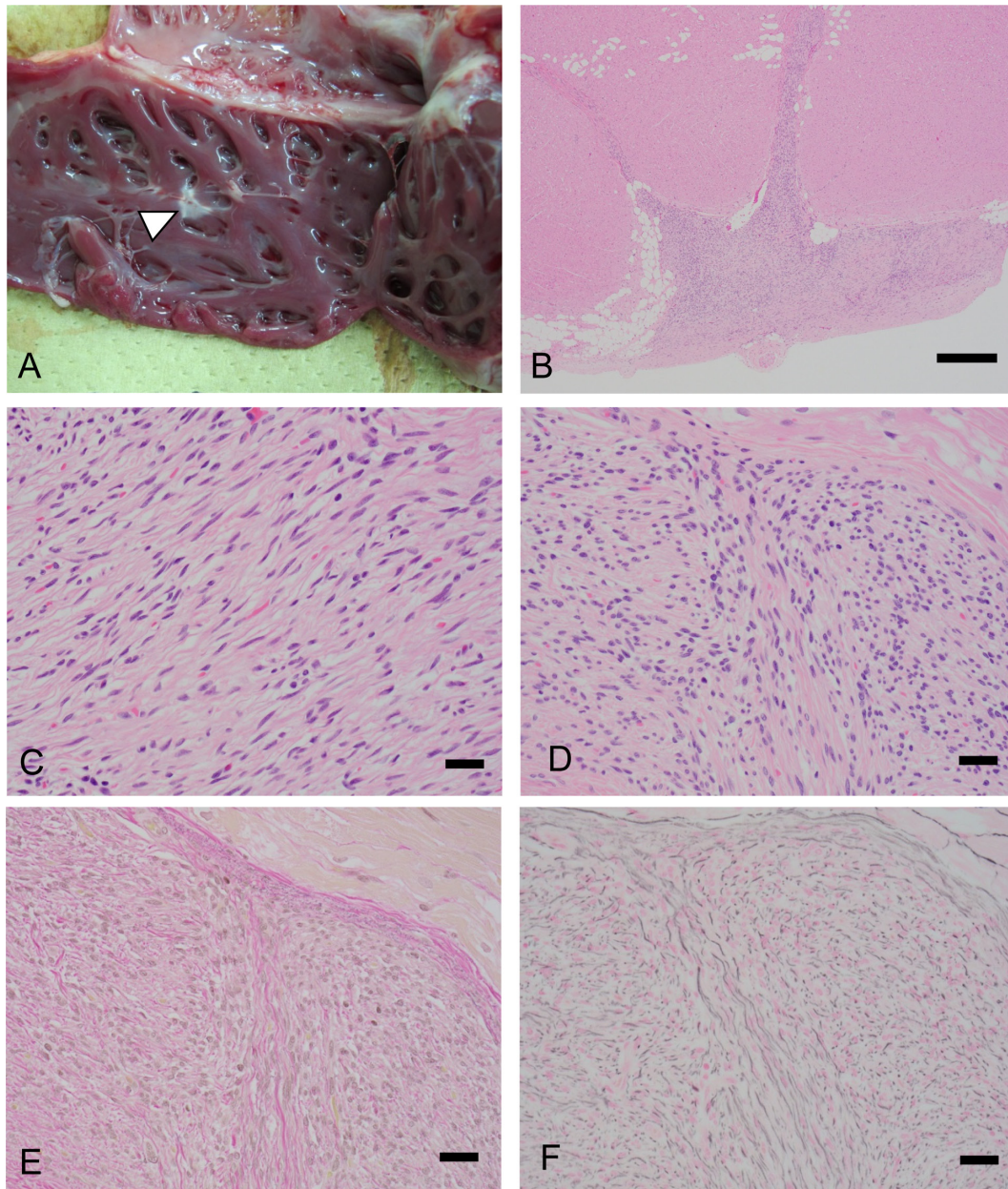
Fig. 1. A: A smooth white focus in the right ventricular endocardium (arrow head). B: The macroscopic lesion with a well-demarcated proliferative lesion expanding the right ventricular endocardial surface (Bar: 500 μm). C and D: The nodule comprising spindle cells arranged in intermediate to long streams and forming broad interlacing fascicles. Spindle cells had round, ovoid to elongated hyperchromatic nuclei or nuclei with finely stippled chromatin and indistinct nucleoli with indistinct cell border. However, there were no findings suggesting leiomyoma such as cigar-shaped nuclei. Spindle cells exhibit minimal anisocytosis and anisokaryosis and mitoses are rare (Bar: 50 μm). E: The presence of abundant collagen was also confirmed upon Elastica van Gieson (EVG) staining. Spindle cells in the lesion were stained yellow by EVG stain. (Bar: 50 μm). F: The proliferating cells lacked the well-developed reticulin fibers outlining individual cells seen by silver staining (Bar: 50 μm).

(Fig. 1E). The cytoplasm in the spindle cells of the lesion stained yellow upon EVG staining. Intercellular stroma/fibers appeared partially black upon silver staining; mainly stained blue upon MT staining; and red upon EVG staining. These findings indicate that the stromal matrix was formed by the proliferating spindle cells. However, silver staining did not show the well-developed reticulin fibers outlining individual proliferating cells (Fig. 1F).

The cytoplasm of the spindle cells was weakly positive for S100, positive for α-SMA, calponin, and vimentin, but negative for GFAP and Schwann/2E (Fig. 2A–F). These results indicate that the spindle cells had a smooth muscle or myofibroblast phenotype and some nuclei were weakly positive for PCNA.
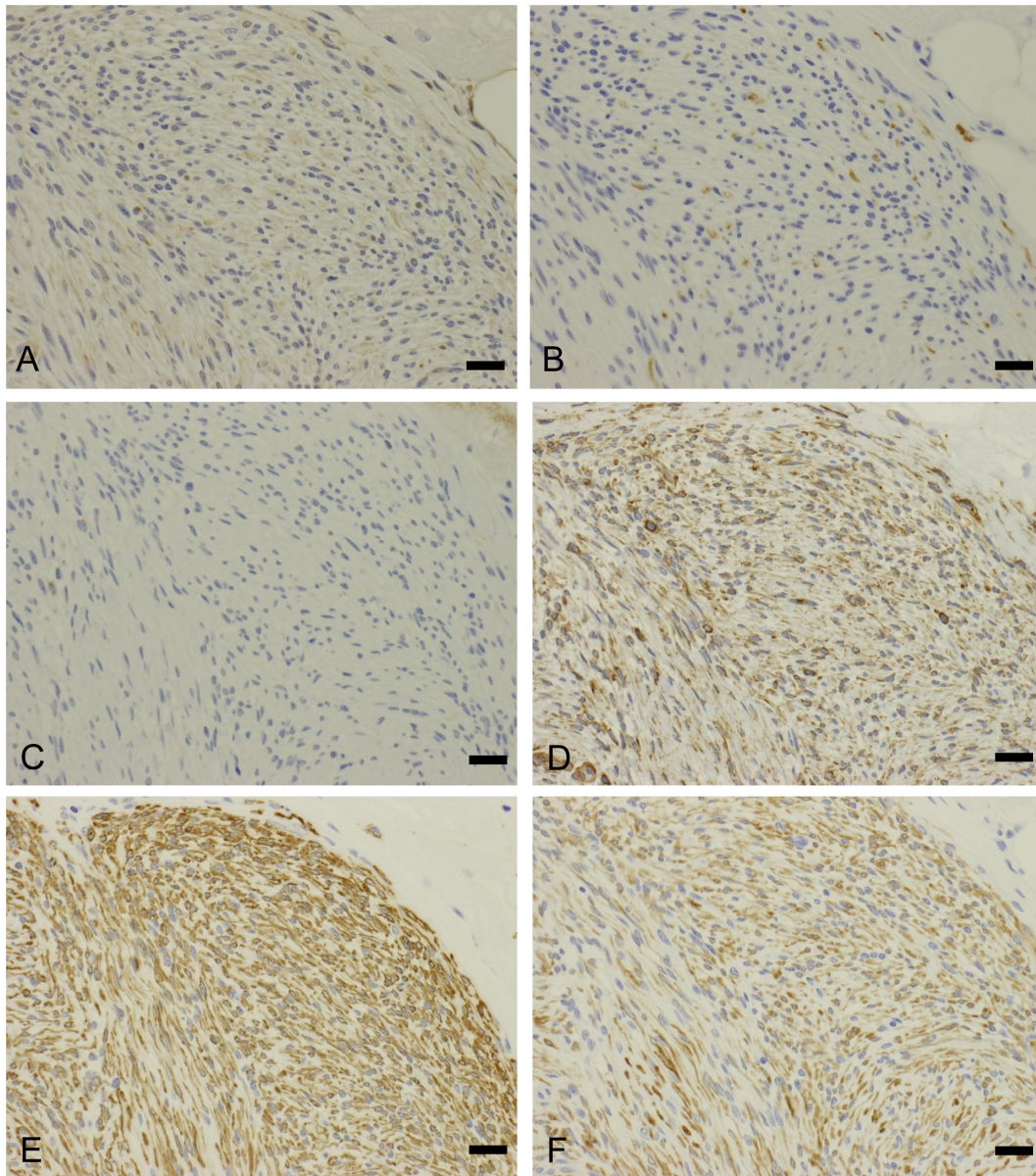
Fig. 2. Immunohistochemical staining of the lesion (Bar: 50 μm). A–C: Cytoplasm of spindle cells was weakly positive for S100 (A) and negative for glial fibrillary acidic protein (GFAP) (B) and Schwann/2E (C). D–F: Cytoplasm of spindle cells was positive for vimentin (D), α-smooth muscle actin (α-SMA) (E), and calponin (F).

The heart tissue comprises cardiomyocytes, cardiac fibroblasts, vascular, and neuronal cells; the endocardium consists of a single layer of endothelial cells, connective tissue, and a rudimentary layer of smooth muscle[4]. Morphologically, the differential diagnosis in this case included hyperplasia of subendocardial Schwann cells, peripheral nerve sheath tumors (endocardial schwannoma, neurofibroma), fibroma/fibrosarcoma, and/or fibrosis. Spontaneous endocardial proliferative lesions that are considered to originate from subendocardial Schwann cells and diagnosed as endocardial schwannomas[2] are well known in rats. However, the presence of α-SMA and calponin are not typical immunohistochemical features of Schwann cells, and thus, hyper-

plasia of Schwann cells and peripheral nerve sheath tumors were ruled out in this case. Cardiac fibroma/fibrosarcoma are rarely reported in dogs and lack α-SMA expression in tumor cells[5–7]. In humans, cardiac fibromas are rare lesions that contain smooth-muscle actin and are typically found in young patients[8]. However, cardiac fibromas are generally known to grossly appear as circumscribed bulging masses; therefore, this nodule was not a fibroma.

Upon immunohistochemical analysis using anti α-SMA and calponin antibodies, we speculated that the nodule likely formed from smooth muscle cells in the endocardium. It has been reported that tumor cells of a canine cardiac leiomyoma exhibit blunt-ended nuclei and are ar-

ranged in intermediate length or long streams and bundles separated by collagen fiber[9]. However, the proliferating cells from the nodule lacked the morphological characteristics of a leiomyoma, such as compressed surrounding normal tissues, well-differentiated smooth muscle cells, cigar-shaped nuclei, and/or well-developed reticulin fibers outlining individual cells; therefore, the possibility of the nodule being a leiomyoma was ruled out.

Based on the histopathological features and differential diagnosis, the proliferating cells were considered to myofibroblasts rather than smooth muscle cells. Myofibroblasts exhibit the characteristics of both fibroblasts and smooth muscle cells and are not normally present in a healthy heart. However, plasticity of cardiac fibroblasts has been reported during tissue remodeling in hypoxic myocardial injuries and these cells can differentiate into myofibroblasts[10]. Moreover, cardiac myofibroblasts can be derived from endothelial cells upon endothelial–mesenchymal transition and from mesenchymal stem cells, bone marrow-derived circulating progenitor cells (fibrocytes), and pericytes[10]. The α-SMA expressing spindle-shaped cells have been identified in experimentally induced myocardial infarctions in rats and also noted in human myocardial scars[11]. In such cases, fibrous granulation tissues are formed along with the surviving cardiomyocytes and/or the endocardium[11]. However, in the present study, proliferating cells with myofibroblast phenotypes were mainly located in the subendocardium and exhibited a characteristic pattern, such as broad interlacing fascicles, without any findings that suggest myocardial necrosis or inflammatory cell infiltration. Therefore, myocardial injury-related fibrosis was ruled out due to the lack of typical histological features.

In this case, we concluded that this lesion was histologically consistent with a proliferative lesion of myofibroblasts. To the best of our knowledge, this is the first report to describe a subendocardial nodular proliferation of myofibroblasts in a dog.

References

1. Treggiari E, Pedro B, Dukes-McEwan J, Gelzer AR, and Blackwood L. A descriptive review of cardiac tumours in dogs and cats. Vet Comp Oncol. **15**: 273–288. 2017. [Medline]

2. Berridge BR, Mowat V, Nagai H, Nyska A, Okazaki Y, Clements PJ, Rinke M, Snyder PW, Boyle MC, and Wells MY. Non-proliferative and proliferative lesions of the cardiovascular system of the rat and mouse. J Toxicol Pathol. **29**(Suppl): 1S–47S. 2016. [Medline]

3. Matsumoto H, and Fukushima R. Primary malignant peripheral nerve sheath tumor of the heart in a dog. Adv. Anim. Cardiol. **49**: 25–28. 2016.

4. Mills SE. Histology for pathologists. 4th ed. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia. 2012.

5. Asakawa MG, Ames MK, and Kim Y. Primary cardiac spindle cell tumor in a dog. Can Vet J. **54**: 672–674. 2013. [Medline]

6. Madarame H, Sato K, Ogihara K, Ishibashi T, Fujii Y, and Wakao Y. Primary cardiac fibrosarcoma in a dog. J Vet Med Sci. **66**: 979–982. 2004. [Medline]

7. Speltz MC, Manivel JC, Tobias AH, and Hayden DW. Primary cardiac fibrosarcoma with pulmonary metastasis in a Labrador Retriever. Vet Pathol. **44**: 403–407. 2007. [Medline]

8. de Montpréville VT, Serraf A, Aznag H, Nashashibi N, Planché C, and Dulmet E. Fibroma and inflammatory myofibroblastic tumor of the heart. Ann Diagn Pathol. **5**: 335–342. 2001. [Medline]

9. Gallay J, Bélanger MC, Hélie P, Côté E, Johnson TO, and Peters ME. Cardiac leiomyoma associated with advanced atrioventricular block in a young dog. J Vet Cardiol. **13**: 71–77. 2011. [Medline]

10. Turner NA, and Porter KE. Function and fate of myofibroblasts after myocardial infarction. Fibrogenesis Tissue Repair. **6**: 5. 2013. [Medline]

11. Willems IE, Havenith MG, De Mey JG, and Daemen MJ. The alpha-smooth muscle actin-positive cells in healing human myocardial scars. Am J Pathol. **145**: 868–875. 1994. [Medline]