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Case Report

A Case Report of a Malignant Fibrous Histiocytoma in a T-cell Receptor β Chain and p53 Double-knockout Mouse

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Abstract: A subcutaneous tumor was found in the right abdomen of a 16-week-old male TCRβ and p53 double-knockout mouse. The tumor had indistinct borders with the surrounding tissue. The cut surface after formalin fixation was pale yellowish white, partially dark red and partly white. Histologically, the tumor was composed of three distinct regions. The first region showed pleomorphic cells arranged in sheets. The second region showed spindle cells arranged in interlacing fascicles. The final region contained a mixture of the above mentioned two types of cells. Furthermore, a small amount of collagen fibers, round cells, multinucleated giant cells, and cells with eosinophilic granules were observed between these tumor cells. Immunohistochemical examination and electron microscopy identified that the pleomorphic cells and spindle cells were histiocytes and fibroblasts, respectively, and that the round cells were undifferentiated mesenchymal cells. Based on these findings, the tumor was diagnosed as a malignant fibrous histiocytoma. (DOI: 10.1293/tox.24.255; J Toxicol Pathol 2011; 24: 251–255)

Key words: malignant fibrous histiocytoma, mouse, TCRβ and p53 double-knockout mouse, spontaneous

A malignant fibrous histiocytoma (MFH) is a tumor that is considered to be derived from pluripotential mesenchymal stem cells. Histologically, it consists of rich fibrous elements and histiocyte-like cells and has various forms. In mice, MFH is composed of proliferating spindle cells and collagen fibers and classified into a fibrous type with a distinctive storiform pattern; a myxoid type, which contains organic material with abundant acid mucopolysaccharide and shows a myxomatous morphology; and a pleomorphic type, which mainly consists of proliferating histiocyte-like cells and contains many multinucleated giant cells[1]. There are reports on MFH or MFH-like sarcoma in A/J mice, ICR mice and genetically modified mice[2,3]. We report here a case of MFH in a 16-week-old male B6.129P2-*TcrbTrp53* (T-cell receptor β chain and p53 double-knockout; TCR $\beta^{-/-}$ $p53^{-/-}$) mouse, in which the spontaneous incidence of colon cancer is high[4,5].

T-cell receptor β chain (TCRβ) is one of the molecules that constitute the T-cell receptor, and lack of this molecule results in homeostatic defects of the intestinal mucosal immune system. The TCRβ gene-deficient ($TCR\beta^{-/-}$) mouse, which has been produced for the purpose of clarifying the mechanisms of T-cell genesis and differentiation[6–9], is known to develop inflammation of the intestinal tract and serves as an animal model of inflammatory bowel disease that exhibits lesions analogous to those of ulcerative colitis in humans[10]. The p53 gene is the cause of Li-Fraumeni syndrome, which is characterized by hereditary, frequent occurrence of diverse malignant neoplasms[11], and is also an antioncogene (tumor suppressor gene) in which mutations frequently are detected in nonhereditary malignant tumors[12]. The protein encoded by p53 functions to arrest the cell cycle in response to DNA damage or to induce apoptosis[13,14]. The p53 gene-deficient ($p53^{-/-}$) mouse, produced to analyze the function of the p53 gene, has been reported to exhibit a high incidence of angiosarcoma and spontaneous development of malignant lymphomas in various organs[15,16].

In the present case, a tumor was found subcutaneously in the abdomen of a male TCR $\beta^{-/-}$ $p53^{-/-}$ mouse. The mouse was housed alone in a plastic cage (182 mm × 260 mm × 128 mm) in an environmentally controlled animal room (temperature, 23 ± 3 °C; relative humidity 50 ± 10%; ventilation rate 10–20 times per hour; and a 12-hour light/12-hour dark cycle) and fed a diet (F-1; Funabashi Farm Co., Ltd., Chiba, Japan) and tap water *ad libitum*. The tumor was observed in the right abdomen of this animal at 14 weeks of age. A hemorrhage developed on the surface of the tumor at 16 weeks of age. The animal was subsequently sacrificed due to general health deterioration.

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The tumor mass was fixed in 10% phosphate-buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin, Masson's trichrome and periodic acid Schiff. Immunohistochemical staining with anti-vimentin mouse monoclonal antibody (DakoCytomation, Kyoto, Japan), anti-factor VIII related antigen rabbit polyclonal antibody (DakoCytomation), anti-S100a protein rabbit polyclonal antibody (DakoCytomation), anti-F4/80 rat monoclonal antibody (Abcam, Tokyo, Japan), anti-alpha smooth muscle actin rabbit monoclonal antibody (Abcam), anti-mouse CD31 rat monoclonal antibody (Abcam), anti-desmin rabbit polyclonal antibody (Lab Vision, Fremont, CA, USA), anti-myoglobin rabbit monoclonal antibody (Epitomics, Burlingame, CA, USA) and anti-Ki-67 rabbit monoclonal antibody (Thermo, Fremont, CA, USA) was performed by the streptavidin-biotin complex method.

For electron microscopic examination, pieces of the 10% formalin-fixed tumor mass were first immersed in phosphate-buffered 2.5% glutaraldehyde and then in phosphate-buffered 1% osmium tetroxide for 2 h. After dehydration through a graded ethanol series, the tissue samples ware embedded in Epon812 resin. Ultrathin sections were prepared and stained with uranyl acetate and lead citrate and then observed under a transmission electronmicroscope (JEM-1200EX; JEOL, Tokyo, Japan).

At necropsy, a 25 × 25 × 13-mm tumor mass was found in the subcutaneous tissue in the right abdomen. Ulceration and hemorrhage were observed on the surface of the tumor mass, and the tumor margin was not clearly visible macroscopically. The cut surface of the formalin-fixed tumor mass was yellowish-white, partly dark red and partly white.

Histological examination showed that the tumor occupied the subcutaneous dermis and that there were many foci of necrosis and hemorrhages in the tumor mass. In addition, ulceration was seen in some parts of the epidermis. The tumor was composed of three distinct regions. The first region showed pleomorphic cells arranged in sheets. The second region showed spindle cells arranged in interlacing fascicles. The final region contained a mixture of the above mentioned two types of cells. The tumor mass also contained hemangiopericytoma-like structures (Fig. 1A, B, C), and disseminated collagen fibers, round cells, multinucleated giant cells and other neoplastic cells containing various numbers of cytoplasmic eosinophilic granules. The pleomorphic cells had an abundant eosinophilic cytoplasm and an atypical nucleus with a prominent nucleolus, and some cells showed phagocytosis of cellular debris. The spindle cells had an eosinophilic cytoplasm and an oval nucleus. Masson's trichrome staining showed a small amount of blue-staining collagen fibers between the cells. The round cells scattered in the tumor contained an eosinophilic cytoplasm and a distinct nucleus. These cells showed a high N/C ratio and frequent mitoses, and some cells showed PAS-positive eosinophilic coarse cytoplasmic granules (Fig. 2A–G).

The pleomorphic cells were immunohistochemically positive for vimentin, F4/80 and Ki-67 (Fig. 2B), while they were negative for S-100a, desmin, α-SMA, myoglobin, factor VIII related antigen, and CD31. The spindle cells were positive for vimentin and Ki-67 and negative for S-100a, F4/80, desmin, α-SMA, myoglobin, factor VIII related antigen, and CD31. The round cells showed similar staining to that of the spindle cells, and the multinucleated giant cells showed the same staining as the pleomorphic cells. The cells with eosinophilic granules were positive for vimentin alone and negative to all other antibodies.

Electron microscopy showed that the pleomorphic cells had phagosomes and lysosomes containing cellular debris (Fig. 3A), a well-developed rough endoplasmic reticulum (r-ER) and Golgi apparatus in the cytoplasm. Abundant free ribosomes and r-ER were observed in the cytoplasm of the spindle cells, and the lumen of some r-ERs were expanded. These cells had an oval nucleus and a distinct nucleolus and the nuclear membrane was deeply stained. Furthermore, fibril formation was also seen in the extracellular spaces (Fig. 3B). The round cells had a high N/C ratio and contained abundant free ribosomes, a few mitochondria and undeveloped r-ERs in the small cytoplasm (Fig. 3C). In the cytoplasm of the cells with eosinophilic granules, several osmiophilic globules were surrounded by a limiting membrane with a diameter of 1 to 1.5 μm, and some of the osmiophilic globules contained glycogen granule-like substances and granules measuring approximately several hundred nm (Fig. 3D).

The tumor mainly consisted of three types of cells, including pleomorphic cells, spindle cells and round cells. Based on the findings of phagocytosis of cellular debris, positive immunohistochemical staining for F4/80 and vimentin and ultrastructural evidence of phagosomes and lysosomes containing cellular debris in the cytoplasm, the pleomorphic cells were suggested to be histiocytes. On the other hand, the spindle cells demonstrated fasciculation, collagen fibers were seen on Masson's trichrome staining and there was ultrastructural evidence of fibril formation. Furthermore, the cells were positive for vimentin on immunohistochemical staining. The above-mentioned findings suggested that the spindle cells might be fibroblasts. It was considered that the round cells scattered in the tumor might be undifferentiated mesenchymal cells because of their positive reaction to vimentin on immunohistochemical staining and no ultrastructural evidence of differentiation to other mesenchymal cells. Thus, the tumor mass in this case consisted of pleomorphic histiocytes, cells undergoing differentiation to fibroblasts and undifferentiated mesenchymal cells, and the tumor was therefore diagnosed as a MFH.

In this case, some tumor cells contained PAS-positive eosinophilic granules that were found to be osmiophilic globules surrounded by a limiting membrane with a diameter of 1 to 1.5 μm on electron microscopy. Some of the globules contained glycogen granule-like substances and granules measuring approximately several hundred nanometers. Cells with these features are called eosinophilic globule (EG) cells, which were reported in a previous study in 18% (27 of 150) of ICR mice with MFH-like sarcoma[3]. The present study could not clarify the causes and signifi-

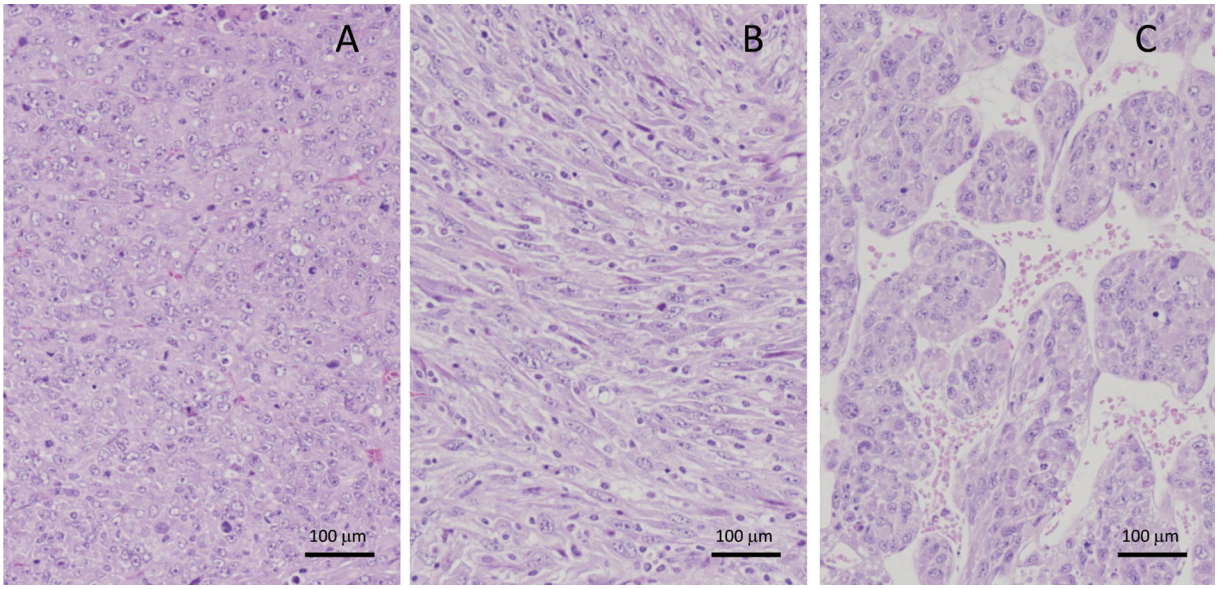

Fig. 1. Histopathological findings of the subcutaneous tumor in a T-cell receptor β chain and p53 double-knockout mouse. Lower magnification of the tumor. A) Pleomorphic cells arranged in sheets. H-E. B) Spindle cells arranged in interalcing fascicles. H-E. C) Hemangiopericytoma-like area.

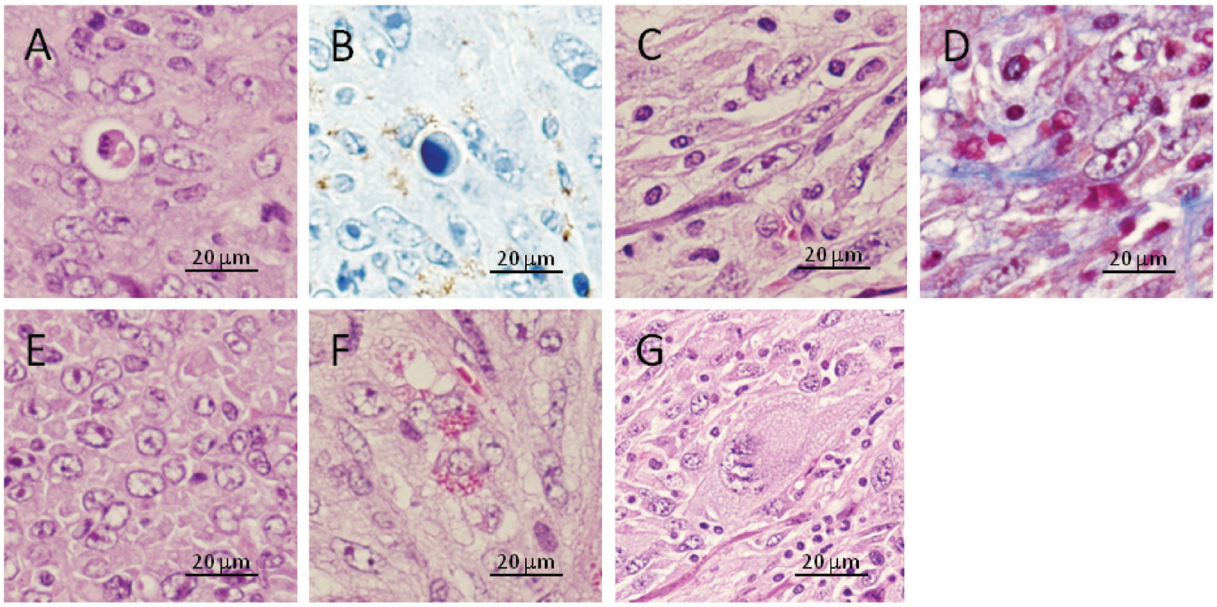

Fig. 2. High magnification of the tumor cells. A) Phagocytosis of cell debris by a pleomorphic cell. H-E. B) Pleomorphic cells showing positive immunohistochemical staining for F4/80. C) Spindle cells. H-E. D) Scanty collagen fibers by the side of spindle cells. M-T. E) Round cells. H-E. F) Eosinophilic globule cells. H-E. G) A multinucleated giant cell. H-E.

cance of production of EG cells but suggested that EG cells are sometimes found in MFH in mice.

The present case did not show proliferation of collagen fibers with typical storiform patterns, which is one of the distinctive features of MFH, but showed a lot of undifferentiated mesenchymal cells, which suggested that it was a relatively poorly differentiated MFH. The incidence of undifferentiated sarcoma in p53 knockout mice was reported to be 14% (8 of 56 mice) by Okada *et al*., 10% (3 of 30 mice) by Harvey *et al.* and 5% (3 of 60 mice) by Donehower *et al*.[15–17]. However, there are no reports of undifferentiated sarcoma in TCRβ knockout mice. Therefore, the relatively poorly-differentiated histology of this case might be attributable to a p53 gene defect.

This case developed MFH at 16 weeks of age, which was a relatively young age. Previous studies reported that

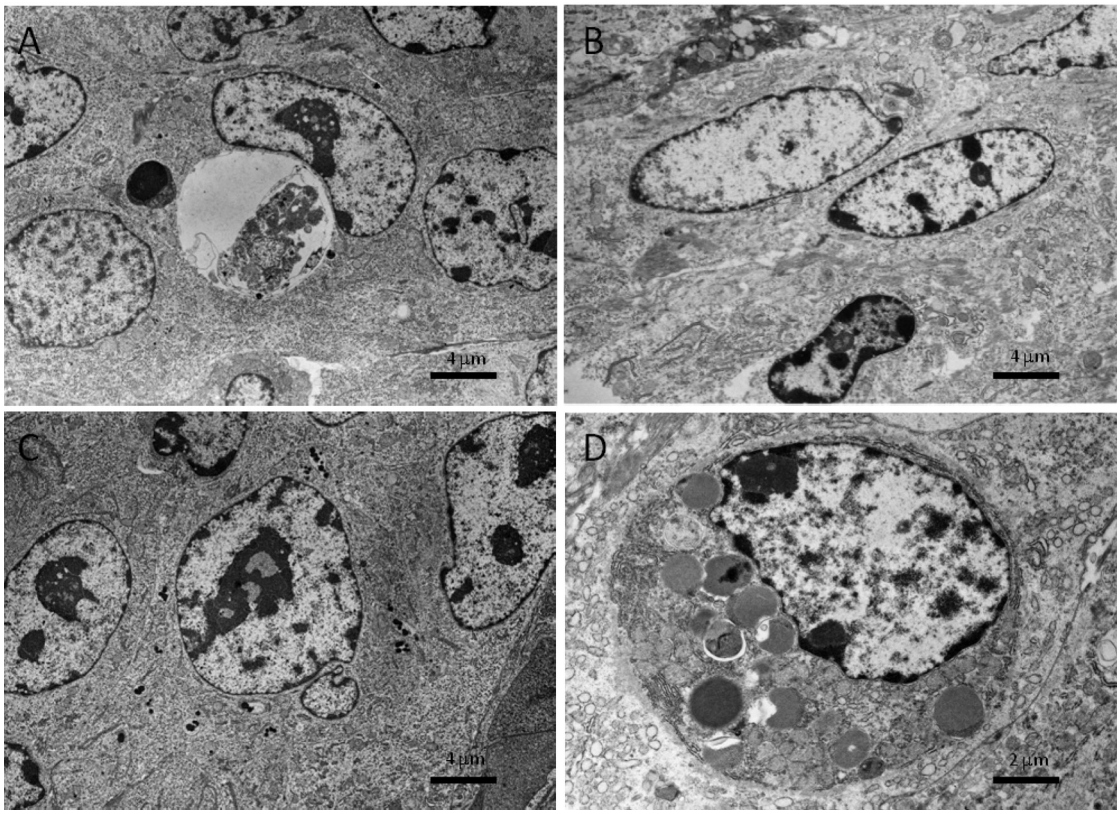

Fig. 3. Electron microscopic findings of tumor cells. A) Phagocytosis of cell debris by a pleomorphic cell with a phagocytic vacuole and lysosome in the cytoplasm. B) The spindle cell producing collagen fibers. C) The round cell had a high N/C ratio. D) An eosinophilic globule cell contained an osmiophilic globule in the cytoplasm.

the age of onset of spontaneous MFH in nontransgenic mice including A/J mice and ICR mice was 1 year old or older[2,3]. Spontaneous MFH has been reported in genetically modified animals including p53 knockout mice and p53 and transporter for antigen presentation-1 double-knockout mice, but the reports did not give the age of onset[17,18]. The life span of p53 knockout mice is approximately 6 months, which suggests that the age of onset of MFH in the above-mentioned genetically modified mice may be less than 6 months old. In addition, various types of sarcoma including malignant lymphoma develop in younger p53 knockout mice under 6 months old[15–18]. The above-mentioned findings suggested that the relatively younger age of onset, 16 weeks, might be attributable to a p53 gene defect.

Many studies have been conducted using various genetically modified mice including p53 gene knockout mice to elucidate the mechanism of pathogenesis and to establish preventive methods. This case indicated that genetically modified mice develop tumors at an early age and that the histological features are different from those previously reported for nontransgenic mice. Accordingly, this case should be taken into consideration when tumors of such genetically modified mice are diagnosed.

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