Subcutaneous Leiomyosarcoma in a Smad3+/- Mouse

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ABSTRACT
Subcutaneous leiomyosarcoma is a malignant tumor that originates from smooth muscles in the skin. Herein, we report a case of subcutaneous leiomyosarcoma that occurred in the ear pinna of a 19-month-old male Smad3+/- mouse. On gross examination, the mass extended to the whole ear auricle and obscured the ear opening. The tumor was approximately 10x10 mm in size, ill-circumscribed, and firm with superficial hemorrhage. The cut surface of the mass was grayish-white and solid with focal necrosis and hemorrhage. Histologically, spindle tumor cells with cigar-like nuclei and plump cells with round nuclei formed broad interlacing fascicles. Additionally, the tumor cells invaded adjacent muscles. Immuno-histochemically, the tumor sections had positive reactions for desmine and α-SMA and were partially positive for vimentin while negative for S-100 and myogenin. Based on the results of the examination, we diagnosed this case as subcutaneous leiomyosarcoma.

Key words:
Ear auricle
Leiomyosarcoma
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Subcutaneous

INTRODUCTION
Leiomyosarcomas are uncommon malignant neoplasms that arise from smooth muscle and comprise approximately 7% of soft tissue sarcomas (Lee et al. 2013). In vertebrates, leiomyosarcoma relatively often occur in the gastrointestinal tract and female genital tract, whereas a few cases of leiomyosarcoma in other organs, such as the oral cavity, esophagus, and urinary bladder have been reported (Nakamura et al., 2010). Superficial leiomyosarcoma is an extremely rare malignant neoplasm arising in the dermis or subcutis in the skin and occurs most frequently in the low extremities (50-70%), followed by the upper extremities (20-30%), the trunk (10-15%), and the facial region (1-5%) (Park et al., 2010). They can be subdivided into two types depending on their location; subcutaneous leiomyosarcomas originate from the smooth muscle cells lining the blood vessels and cutaneous leiomyosarcomas originate from the arrector pilae muscle of the hair follicles or the dartos muscles of the genital skin (Torres et al., 2011). Some reports describing subcutaneous leiomyosarcomas in humans, dogs, cats, ferrets and monkeys (Nakamura et al., 2010; Park et al., 2010). But, a subcutaneous leiomyosarcoma occurring in the face of a laboratory mouse has not been reported before.

Smad3, downstream mediator of TGFβ1, has an important role in cell cycle and tumor suppression (Brown et al., 2008). Smad3 transgenic mice have played an important role and been widely studied in various research fields. Here, we present a case of subcutaneous leiomyosarcoma that occurred on the ear of a Smad3+/- mouse.

MATERIALS AND METHODS
The Smad3 transgenic mice were acquired from the National Cancer Institute, MD, USA. The mice were kept in a room at 22±2°C with 50±10% relative humidity on a constant photoperiod and were fed standard laboratory feed and water ad libitum. After the 19-month-old ear tumor presenting male Smad3 transgenic mouse died, its genotypes were confirmed by PCR analysis using primer 1 (5’-CCACTTCATTGCCATATGCCCTG-3’), primer 2 (5’-CCGGAACAGTGGATTCACACA-3) and primer 3 (5’-CCAGACTGCCTTGGGAAAAGC-3’). The primer 1 and 2 pair amplifies a fragment of 481 bp from the Smad3 wild type allele. The 284 bp fragment amplified with
Fig. 1: Photographs of ear mass (A, B) and PCR analysis of Genomic DNA extracted from the examined mice. Before excision, it was shown that the mass extended to the whole ear auricle and obscured the ear opening (A). The cut surface of the mass was approximately 10x10 mm in size, grayish-white and solid with focal necrosis (arrow head) and hemorrhage (arrow) (B). The Smad3 +/- mice showed 284bp and 431bp PCR bands (C).

Fig. 2: Microscopic photographs. (A) The plump ovoid cells with round nuclei and the spindle cells with cigar-like nuclei were shown. Many clear vacuoles and little mitotic figure were observed among the tumor cells. HE. Scale bars = 50µm; (B) The ovoid cells and the spindle cells formed broad interlacing fascicles. HE. Scale bars = 100µm; Invasion of the tumor cells to adjacent skeletal muscle were observed (Insert of Fig. 2B).

Fig. 3: Microscopic photographs of immunohistochemistry (A-C). (A) there was a strongly positive reaction for desmin. Scale bar = 50µm; (B) strongly positive reaction for α-SMA. Scale bar = 50µm; (C) weakly positive reaction for vimentin. Scale bar = 50µm

primers 1 and 3 represents the Smad3 mutant type allele (Jeong et al., 2008).

For histological examination, the mass was rapidly excised and immediately fixed in 10% neutral-buffered formalin for 24 hours before being routinely processed and embedded in paraffin wax. The embedded samples were sectioned to 4 µm thicknesses. The sectioned tissues were stained with hematoxylin and eosin (H&E), and then, immunohistochemistry (IHC) was performed using an avidin-biotin-peroxidase complex method (Invitrogen, MD, USA) with 3, 3-diaminobenzidine (Invitrogen, USA) for differential diagnosis. IHC with various primary antigens including anti-vimentin (DakoCytomation, USA), anti-α-smooth muscle actin (Abcam, USA), anti-desmin (DakoCytomation, USA), anti-myogenin (Abcam, USA) and anti-S100 (Santa Cruz Biotechnology, USA) was routinely performed. The tissue sections were also subjected to Masson’s trichrome staining, to check for the presence of collagen fibers in tumor mass.

RESULTS AND DISCUSSION

The Smad3 transgenic mouse had a white mass on its left ear pinna. Gross examination revealed that the mass was approximately 10x10 mm in size and ill-demarcated with a firm mass and superficial hemorrhage. The auricular mass occupied the whole ear pinna and blocked the ear opening entirely (Fig. 1A). The cut surface of the mass was grayish-white and solid with focal necrosis and hemorrhage (Fig. 1B). No remarkable lesion was observed in other organs. From the histological examination, the tumor was non-encapsulated and densely composed of pleomorphic plump ovoid to spindle cells with eosinophilic cytoplasm (Fig. 2A). The spindle cells with
cigar-shaped nuclei and the plump ovoid cells with round nuclei formed broad interlacing fascicles (Fig. 2B). Mitotic figures were observed, ranging from zero to two at x400 magnification. Invasion of the tumor cell to adjacent skeletal muscle (Fig. 2B), loss of skin adnexa, and inward epithelial hyperplasia were observed. However, from Masson's trichrome staining, collagen fibers were not observed among the tumor cells. From the IHC staining, the tumor sections were strongly positive for desmin (Fig. 3A) and a-SMA (Fig. 3B) and partially positive for vimentin (Fig. 3C) while negative for S-100 and myogenin.

The initial diagnosis followed by the H&E staining examinations showed that spindle-shaped cells were cutaneous spindle cell tumor. The differential diagnoses of cutaneous spindle cell tumor are spindle cell squamous cell carcinoma, desmoplastic melanoma, dermato-fibrosarcoma protuberans, undifferentiated pleomorphic sarcoma, atypical fibroxanthoma, myofibrosarcoma, and superficial leiomyosarcoma (Fauth et al., 2010; Hollmig et al., 2012). Based on the IHC staining, all candidate neoplasms except for myofibrosarcoma and superficial leiomyosarcoma were ruled out. Because both myofibrosarcoma and superficial leiomyosarcoma are positive for desmin, a-SMA and vimentin, Masson's trichrome staining was needed to confirm whether collagen fibers existed among the tumor cells. (Hollmig et al., 2012). Following the absence of collagen fibers, myofibrosarcoma was completely ruled out.

Superficial leiomyosarcoma is subdivided into cutaneous and subcutaneous leiomyosarcoma according to its primary site of origin. Subcutaneous leiomyosarcoma originates from the smooth muscle cells lining the blood vessels and cutaneous leiomyosarcoma originates from the arrector pili muscle of the hair follicles or the dartos muscles of the genital skin (Torres et al., 2011). In this case, the tumor cells that originated from the smooth muscles in the walls of blood vessels in the subcutaneous tissue were observed and showed invasion toward the adjacent dermal layer and skeletal muscles. Consequently, considering the results of all those examinations, the present case was diagnosed as subcutaneous leiomyosarcoma.

Smad3, a downstream mediator of TGFβ1, has an important role in cell cycle, wound healing, and tumor suppression (Brown et al., 2008). In Smad3 knockout mice, deletion of Smad3 accelerates wound healing by keratinocyte migration and proliferation with matrix support, whereas the wound occurring in the ear was enlarged due to absence of the matrix support (Arany et al., 2006). In this case, we could not determine the relationship between the tumorigenesis of smooth muscles and the Smad3 deficiency. To elucidate how the Smad3 deficiency affects the tumorigenesis of smooth muscles, additional studies need to be farther step.

Superficial leiomyosarcoma is an extremely rare leiomyosarcoma, which originates from smooth muscles in blood vessels and the arrector piliae muscle of hair follicles in the dermis and/or subcutis (Park et al., 2010). Moreover, superficial leiomyosarcoma that occurs on the face accounts for just 1-5% of neoplasms (Lin and Tsai, 1999). Very few cases of subcutaneous leiomyosarcoma have been reported until now in laboratory animals. Additionally, in the case of ear auricular tumors, a relatively small number has been reported. To the author's knowledge, this case is the first report of a facial subcutaneous leiomyosarcoma in a laboratory animal and has significance because of its uniqueness.

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REFERENCES