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Spontaneous Multicentric Malignant Schwannoma in a Male Fischer 344 Rat

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We describe here a multicentric spontaneous malignant schwannoma obtained from one male F344 rat, and this animal was the subject of a carcinogenicity study for which it was treated with diisodecyl phthalate. The animal of the control group not treated with diisodecyl phthalate showed dyspnea and severe lordosis. On the necropsy, two tan, firm, encapsulated masses were observed in the subcutis of the lumbosacral region and the left inguinal region of the abdominal cavity, respectively; the masses were 25 × 17 × 8 mm and 16 × 14 × 8 mm in size, respectively. Histologically, the tumor consisted of spindle and pleomorphic cells that grew in various patterns, that was, sweeping fascicles and herringbone and local organoid patterns. The pleomorphic neoplastic cells had more than two nuclei. Additionally, the diagnosis of malignant schwannoma was confirmed by the immune reactivity of the tumor cells for S-100 protein.

***Key words*:** Multicentric spontaneous malignant schwannoma, F344 rat, S-100 protein

INTRODUCTION

Di-isodecyl phthalate (DIDP), a peroxisome proliferator-activated receptor-alpha activator, is widely used as a plasticizer in the manufacture of polyvinyl chloride (PVC), particularly wire, cable and toys, etc. In the carcinogenecity studies, DIDP has no carcinogenic potential in F344 rat (Cho *et al*., 2008). The non-carcinogenicity of DIDP in F344 rats was due to its limited potential for peroxisomal proliferating activity.

Schwannoma is a neoplasm that originates from Schwann cells of a neural sheath; it is most commonly seen in the subcutis of the flank or the neck area near the salivary glands (Laber-Laird *et al*., 1988; Sharma *et al*., 1990; Colmenero *et al*., 1991; Yoshida, 1992). In rats, schwannoma generally occurs in the thoracic and abdominal cavities, spinal cord, cranial cavity and heart, but the incidence of spontaneous malignant schwannoma is very low (0.3%) (Haseman *et al*., 1998). We described here a case of spontaneous malignant schwannoma with multiple histological characteristics, and this tumor involved the subcutis and the abdominal cavity of a male F344 rat that was used in a 2-year carcinogenicity study of diisodecyl phthalate (DIDP).

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MATERIALS AND METRHODS

Specific pathogen free F344/DjCrj rats were obtained from Charles River Japan, Inc., and they were housed in a polycarbonate cage with hardwood bedding materials. The animals were fed commercial mouse pellets (Biogenomics Co.) and water *ad libitum*. The environmental conditions were controlled with the ambient temperature set at 23 ± 2°C, the relative humidity was set at 50 ± 5% and the lightening was set at a 12-hr cycle. They were fed DIDP in their diet at the levels of 0, 0.04, 0.2 or 0.8% (w/w) for 104 weeks. We conducted regular checks on their clinical signs. In this study, we found tumor masses in a male rat at age of 110 weeks in the control group not treated with DIDP.

For histopathological examination, all the tissues from the masses were fixed in 10% neutral phosphate buffered formalin; they were processed in a routine manner, paraffin embedded and then stained with hematoxylin-eosin (HE), Fontana-Masson's argentaffin, Masson's trichrome and Periodic acid-Schiff's (PAS). Additionally, the masses were immunostained for vimentin (DAKO, Glostrup, Denmark), glial fibrillary acidic protein (GFAP) (DAKO, Glostrup, Denmark), neuron specific enolase (NSE) (Biomeda, CA, USA), S-100

protein (DAKO, Glostrup, Denmark), desmin (DAKO, Glostrup, Denmark) and smooth muscle cell actin (DAKO, Glostrup, Denmark) with using a rabbit ABC Staining System (Santa Cruz Biotechnology, Inc., California, US). The deparaffinized sections were quenched in 3% hydrogen peroxide in methanol for 10 min prior to incubation with the appropriately diluted antibodies: 1 : 400 for GFAP, S-100 protein and desmin, 1 : 100 for vimentin and smooth muscle cell actin, and the NSE was commercially prediluted.

RESULTS AND DISCUSSION

One male rat of the control group showed dyspnea, emaciation and severe lordosis of the lumbar vertebrae at the post initiation 16th week of the study. At necropsy, the 5 and 6th lumbar vertebrae were severely lordotic. Two tan, firm, encapsulated masses were observed in the subcutis of the lumbosacral region (25 × 17 × 8 mm) (Fig. 1A) and the inguinal region of the abdominal cavity (16 × 14 × 8 mm), respectively (Fig. 1B). The masses were well separated from the surrounding tissues and they had gray to red foci on their cut sections.

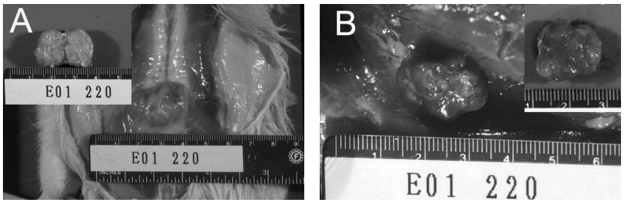

Fig. 1. Gross findings of the malignant schwannoma. Note the two tan, firm, encapsulated masses in the subcutis of the lumbosacral region (25 × 17 × 8 mm) (**A**) and the inguinal region of the abdominal cavity (16 × 14 × 8 mm) (**B**).

Histologically, multiple cell growth patterns were observed and the two masses showed similar morphological findings. Both of them were composed of highly cellular spindle-shaped cells with an Antoni A pattern. They were deposited in sweeping fascicles (Fig. 2A) as stiff, straight cells arrayed in a herringbone pattern (Fig. 2B). The tumor cells were also infrequently arranged in an organoid growth pattern in which the cell nuclei were plump and the cell processes radiated toward a center (Fig. 2C). The tumor cells were fusiform-shaped, they had hyperchromatic oval nuclei and moderate amounts of faintly eosinophilic cytoplasm (Fig. 2D), and mitotic features were frequently seen. In addition, the tumor masses showed extensive necrosis (Fig. 2E).

On immunohistochemistry, the neoplastic cells showed positive staining for vimentin, GFAP and NSE throughout the masses, and patchy staining (both positive and negative staining) was seen for S-100 protein (Fig. 3). However, they were negative for desmin and smooth muscle cell actin. They were also not reactive on PAS staining and argentaffin staining, and they didn't possess a thick hyaline capsule, a feature that was highlighted with trichrome stains (Fig. 3). Therefore, the masses were diagnosed as malignant schwannoma, based on the results of the microscopic examination, the immunohistochemistry and the special stains.

Most schwannomas consists entirely of Schwann cells arranged in two characteristic patterns, which are referred to as Antoni A and B (Laber-Laird *et al*., 1988; Rice and Ward, 1998). The Antoni A pattern features compactly aligned

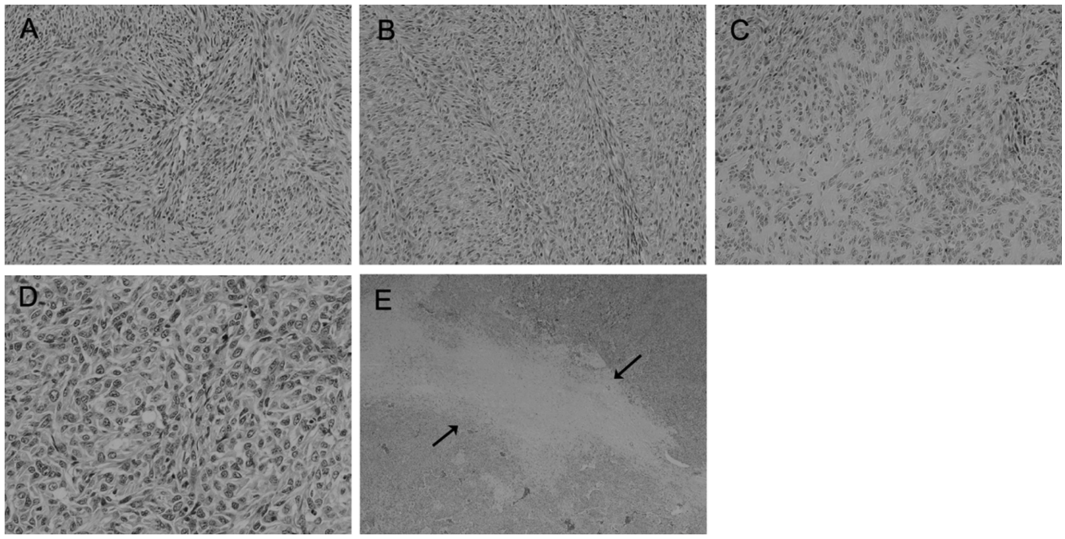

Fig. 2. Malignant schwannoma. The majority of the tumor was composed of spindle cells disposed in sweeping fascicles (H&E. ×100) (**A**) and in a herringbone pattern (H&E. ×100) (**B**). A minority of cells was arranged in an organoid growth pattern, and the nuclei of these cells were plump and the cell processes radiated toward a center (H&E. ×100) (**C**). The tumor cells exhibit marked nuclear pleomorphism with frequent mitoses (H&E. ×200) (**D**). The masses showed diffuse necrosis (arrows) (H&E. ×40) (**E**).

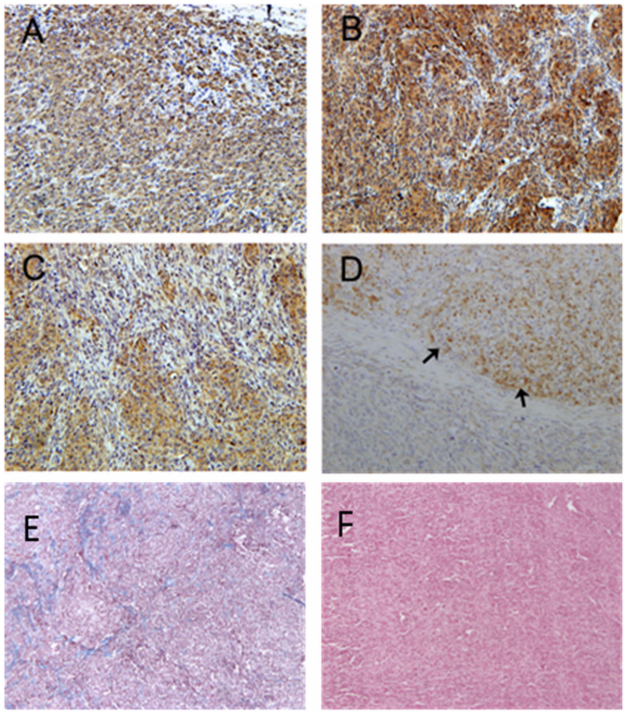

Fig. 3. A malignant schwannoma immunohistochemically stained for vimentin (×100) (**A**), glial fibrillary acidic protein (GFAP, ×100) (**B**), neuron specific enolase (NSE, ×100) (**C**) and positive reaction (arrows) of S-100 protein (×100) (**D**). Negative reaction of Masson's Trichrome (×100) **(E)**, Negative reaction of Fontana Masson's argentaffin staining (×100) **(F)**.

spindle cells with elongate nuclei, while the Antoni B pattern consists of loose textured cells with round nuclei and delicate cobweb-like processes. In this study, both masses in the inguinal and the lumbosacral regions showed histological findings of Antoni type A. Based on the normal results the histopathology of the lymph nodes, we can guess the two masses occurred independently, not metastatically.

Staining for S-100 protein is useful for identifying schwannomas (Weiss *et al*., 1983; Kawahara *et al*., 1988), although it is a nonspecific marker of nerve sheath tumors (Johnson *et al*., 1988; GianGaspero *et al*., 1989; Gray *et al*., 1989). Here, we observed a patchy S-100 staining pattern. This not only excluded various nonneural soft tissues, but it is also one of the characteristics that can distinguish malignant schwannoma from benign schwannoma; benign schwannomas usually have a uniform positive immunoreactivity for S-100 protein, while malignant schwannomas have both positive and negative immunoreactivity (Weiss *et al*., 1983; Rice and Ward, 1988). On the other hand, benign schwannomas possess a thick hyaline capsule and considerable collagen deposition; these features are highlighted with trichrome stains and the schwamnnoma cells and the capsule and collagen deposits form tight, occasionally discrete aggregates termed Verocay bodies, and these Verocay bodies show strong PAS staining. Therefore, the negative results of trichrome and PAS staining support that these masses were malignant schwannoma. Furthermore, the masses characteristically displayed diffuse necrosis, which is one of evidences that could be used to diagnose malignant schwannoma (Hruban *et al*., 1990).

Other possible diagnoses were neurofibroma, fibrous histiocytoma, hemangiopericytoma, melanoma and so on. However, we could easily rule them out because the masses showed positive immunoreactivity for S-100 and NSE, a negative result for Fontana-Masson's argentaffin staining (Fig. 3) and there was no vascular proliferation or severe hemorrhage.

Despite that the incidence rate of spontaneous peripheral nerve sheath tumors in F344 rats is known to be low (0.05%) (Goodman *et al*., 1979; Yoshida, 1992), we were able to observe multicentric spontaneous malignant schwannoma with histological findings of various Antoni type A. And this tumor is not the effects of test substance because the lesion was found in the control group of the carcinogenicity study. Therefore, this report may provide a good reference on spontaneous malignant schwannoma in 2-year carcinogenecity studies, although a further study needs to be conducted to obtain the electron microscopy findings.

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