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Postoperative fibromatosis-type fibromas in the *Bhd* gene mutant (Nihon) rat

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Abstract

Fibromatosis-type fibromas were found to develop at abdominal surgical sites in 4 heterozygous Nihon rats, a model for the human Birt–Hogg–Dubé syndrome. In all 4 rats, solitary and firm nodules were located within the lateral abdominal musculature involving the full thickness of the abdominal wall at the sites of laparotomy. Histologically, the nodules consisted of well-differentiated fibroblastic spindle-shaped cells. These cells were surrounded by large amounts of collagen fibers, and appeared to infiltrate within the abdominal musculature. A portion of the spindle-shaped cells showed features of myofibroblasts. These characteristics are consistent with desmoid tumors in human. Although the etiology of desmoid tumors in human remains unclear, they are known to occur in association with hormonal factors, surgical trauma, and familial adenomatous polyposis. In animals, they have been reported in dogs, cats, horses, and genetically modified mouse models for human familial adenomatous polyposis. The development of the tumors in the Nihon rats was apparently associated with surgical incisions. Genetic factor should be involved in the occurrence of the tumor, since it was found only in the Nihon rats among many rats. Our present data suggest that *Bhd* gene mutation is not likely to be a candidate.

Keywords: Fibroma; Fibromatosis; Desmoid tumor; Postoperative; Nihon rat; Birt–Hogg–Dubé syndrome; BHD

Introduction

Fibromatoses occurring in human consist of 2 major types of superficial (fascial) and deep (musculoaponeurotic) fibromatosis, and are characterized by proliferation of well-differentiated fibroblasts, infiltrative growth pattern, presence of variable amount of collagen, lack of cytological features of malignancy, and aggressive clinical behavior with repeated local recurrences but lack of capacity to metastasize (Enzinger and Weiss, 1995; Kempson et al., 2001). The deep type involves deeper structure, particularly the musculature, and the

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descriptive term "desmoid tumor" is used as a synonym for this type of fibromatosis (Enzinger and Weiss, 1995). Desmoid tumors are rare in humans, the incidence being estimated at only 2.3–4.3 new cases/million/yr (Reitamo et al., 1982). Most cases of these tumors are sporadic; however, there is a clear association with hormonal factors and trauma including surgical incisions (De Cian et al., 1999; Enzinger and Weiss, 1995; Fujita et al., 2003; Hayry et al., 1982; Satsuma et al., 2003). Desmoid tumors also occur in association with familial adenomatous polyposis (FAP) and Gardner's syndrome, suggesting an association with mutations of the *adenomatous polyposis coli* (*APC*) gene on chromosome 5q21–22 (Clark and Phillips, 1996; Giarola et al., 1998; Groden et al., 1991; Kinzler et al., 1991; Klemmer et al., 1987; Knudsen and Bulow, 2001; Rodriguez-Bigas et al., 1994).

In animals, desmoid tumors have been reported to occur in dogs, cats, horses, and genetically modified mouse models for human FAP (Cook et al., 1998; Ihrke et al., 1983; Motozawa et al., 1992; Smits et al., 1998; Valentine et al., 1999). Here we describe fibromatosis-type fibromas that closely resemble human desmoid tumors (deep fibromatosis). The tumors developed in the Nihon rat, a novel rat model we discovered for the human Birt–Hogg–Dubé syndrome (Hino et al., 2001; Kouchi et al., 2006; Okimoto et al., 2000, 2004), an autosomal dominant disease characterized by development of renal cell carcinomas, fibrofolliculomas, trichodiscomas, acrochordon, pulmonary cysts, and/or spontaneous pneumothorax (Birt et al., 1977; Nickerson et al., 2002; Pavlovich et al., 2002; Toro et al., 1999; Zbar et al., 2002). The Nihon rat has a germ-line mutation in 1 allele of the *Bhd* gene where insertion of a single nucleotide results in a frameshift at codon 17 and production of a stop codon 26 aa down stream (Hino et al., 2001; Okimoto et al., 2000, 2004). Renal cell carcinomas are induced in a hereditary manner through "Knudson's two-hit" process in the Nihon rat (Okimoto et al., 2000, 2004). Homozygotes of the germ-line mutation of the *Bhd* gene demonstrate lethality during the early stage of pregnancy (Okimoto et al., 2000).

Our present data representing the fibromatosis-type fibroma in rats suggest that the tumors in this rat model are unlikely derived from *Bhd* gene mutation, but rather develop in association with surgical incisions. However, any involvement of genetic factor(s) is not conclusively excluded.

Materials and methods

Procedures for animal care and housing were in compliance with the institutional guidelines for the care and use of laboratory animals. All animal experiments were performed under protocols approved by the Institutional Animal Care and Use Committee of Dainippon Sumitomo Pharma Co. Ltd.

In total, 340 rats obtained from a colony of the Nihon rats were assigned at 4 weeks old to 3 long-term studies consisting of 215, 66 and 59 rats, respectively, for investigation of hereditary renal cell carcinomas. All rats were maintained in a barrier facility for up to 70 weeks. Among them, 161 rats underwent laparotomy of the lateral abdomen when they were 10 weeks old under sodium pentobarbital anesthesia (50 mg/kg body weight, i.p.) to ascertain the presence or absence of early neoplastic lesions in the kidneys. These procedures differentiated the Nihon rats (heterozygotes) from wild-type rats since phenotypic expression has been assured to be consistent with genotypic expression (Hino et al., 2001). All the rats were subjected to gross and histological examinations when they were found dead or *in extremis* and at the end of the study period where the surviving rats were anesthetized with sodium pentobarbital (50 mg/kg body weight, i.p.) and exsanguinated prior to necropsy. For the 4 rats that developed abdominal nodules, all organs including the nodules and kidneys were fixed in 10% buffered formalin, sliced, and routinely processed for embedding in paraffin. Histological sections were cut at 3 μm, and stained with hematoxylin and eosin (H&E) for all organs, and also with azan staining for the nodules. Immunohistochemical staining was applied to the nodules according to standard procedures using a mouse monoclonal primary anti-human alpha-smooth muscle actin (α-SMA) 1A4 (Dako Japan, Kyoto, Japan) antibody. Small cubes of nodules were fixed in buffered 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in epon resin. Ultra-thin sections were cut, contrasted with uranyl acetate and lead citrate, and examined using a Hitachi 7600 transmission electron microscope (Hitachi High-Technologies Co., Tokyo, Japan). Two (Cases 1 and 2) out of 4 lateroabdominal nodules found in Nihon rats were subjected to genomic analysis as described by Okimoto et al. (2004).

Results

The rats allocated on 3 studies were found to be composed of 227 mutant rats (Nihon rats) and 113 wild-type rats (Table 1). Amongst these animals, lateroabdominal nodules were noted at the sites of laparotomy in 4 male Nihon rats necropsied at the ages of 54, 55, 67 and 69 weeks, respectively, in addition to renal cell carcinomas that were expected to develop in all the Nihon rats (Table 2). The nodules were all solitary and firm, and their gross appearance was similar. They were located within the lateral abdominal musculature at the