

CASE REPORT

Seiichi Ishii · Shuangyin Han · Ken-ichi Shiiba
Takayuki Mizoi · Mitsunori Okabe · Akira Horii
Hiroshi Nagura · Seiki Matsuno · Iwao Sasaki

Allelic loss of the *NF1* gene in anal malignant melanoma in a patient with neurofibromatosis type 1

Received: August 31, 2000 / Accepted: May 11, 2001

Abstract A 64-year-old man with neurofibromatosis type 1 (NF1) developed a primary malignant melanoma of the anus. Genetic analysis of the resected tumor confirmed loss of heterozygosity (LOH) of the *NF1* gene. Anorectal malignant melanoma in NF1 is extremely rare, and genetic studies of the *NF1* gene in such patients have not been reported. The allelic loss detected in the present patient supports the previously raised idea that *NF1* can function as a tumor suppressor gene in the development of malignant melanoma in patients with NF1.

Key words Anal malignant melanoma · Neurofibromatosis · *NF1* · LOH

Introduction

Neurofibromatosis type 1 (NF1), also called von Recklinghausen's disease, is a common autosomal dominant disorder affecting approximately 1 in 3000 to 5000 people in all ethnic groups.^{1,2} The diagnosis of NF1 is currently based on clinical criteria including the presence of multiple café-au-lait spots, neurofibromas, and other fea-

tures.^{3,4} In addition to neurofibroma, it is known that patients with NF1 are at increased risk of the development of both benign and malignant tumors predominantly derived from the neural crest.^{5,6} The gene responsible for NF1, which has been cloned at chromosome 17q11.2, spans more than 350kb and contains 60 exons.^{7–11} It has been suggested that *NF1* functions as a tumor suppressor gene in some types of cells, including normal melanocytes, that are of neural crest origin.^{12–15} However, it is still controversial whether malignant melanoma is more prevalent in patients with NF1 than in the general population.^{16–19} Anorectal malignant melanoma is a rare neoplasia^{20–22} and, to our knowledge, only two cases of the malignancy in patients with NF1 have been reported previously.^{23,24} In this report, we show the somatic loss of an *NF1* allele in anal malignant melanoma that developed in a patient with NF1.

Case report

A 64-year-old Japanese man visited the Tohoku University Hospital in February 1998 because of a prolapsed anal tumor with hemorrhage. He had been diagnosed as having neurofibromatosis type 1 (NF1) because of the presence of multiple café-au-lait spots (Fig. 1) and cutaneous and subcutaneous tumors and neurofibromas of the duodenum. He had undergone pancreaticoduodenectomy for multiple submucosal tumors of the duodenum at age 59 years. He had a family history of gastric cancer (three individuals who were first-degree relatives had been affected); however, none of his family members including his two children, exhibited an NF1 phenotype. Thus, his case was considered to be de novo NF1. A black tumor, 3.2 × 3.0 cm in size, was present on the anterior wall of the anal canal (Fig. 2a), and biopsy revealed malignant melanoma.

Abdominoperineal resection was carried out in April 1998. The tumor arose on the dentate line and infiltrated to the submucosal layer of the anal canal (Fig. 2b) with invasion to the rectal muscularis mucosae. Histopathology of the melanoma revealed dense spindle cells with hyperchro-

S. Ishii (✉) · S. Han · K. Shiiba · T. Mizoi · M. Okabe · I. Sasaki

Division of Biological Regulation and Oncology, Department of Surgery, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan
Tel. +81-22-717-7205; Fax +81-22-717-7209
e-mail: s.ishii@surg1.med.tohoku.ac.jp

S. Han · A. Horii
Department of Molecular Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan

H. Nagura
Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan

S. Matsuno
Division of Gastroenterological Surgery, Department of Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan

matic nuclei, prominent nucleoli, and melanin granules in the cytoplasm (Fig. 3a). The tumor cells were positive for HMB45 (Fig. 3b), but almost negative for S100 protein (Fig. 3c) by immunohistochemistry, with an anti-human melanoma monoclonal antibody, HMB45 (Dako, Glostrup, Denmark), and an anti-S100 protein polyclonal antibody (Dako). Two perirectal lymph nodes showed melanoma cell metastasis.

Loss of heterozygosity (LOH) analysis of the *NFI* gene was carried out, using the polymerase chain reaction (PCR) amplification of a highly polymorphic microsatellite marker, IVS38GT53.0, in intron 38 of *NFI*,²⁵ with the approval of the Ethical Issues Committee of Tohoku University Hospital and the informed consent of the patient. LOH of the IVS38GT53.0 microsatellite allele was detected in the genomic DNA of the malignant melanoma, although DNA



Fig. 1. Multiple café-au-lait spots on the back of the patient

from the peripheral blood cells retained heterozygosity (Fig. 4). LOH at chromosome 9p21, which has been shown to be frequent in sporadic malignant melanoma,²⁶ was also examined with a microsatellite marker, D9S270.²⁷ The analysis was not informative because the patient's genomic DNA was homozygous for the marker (data not shown).

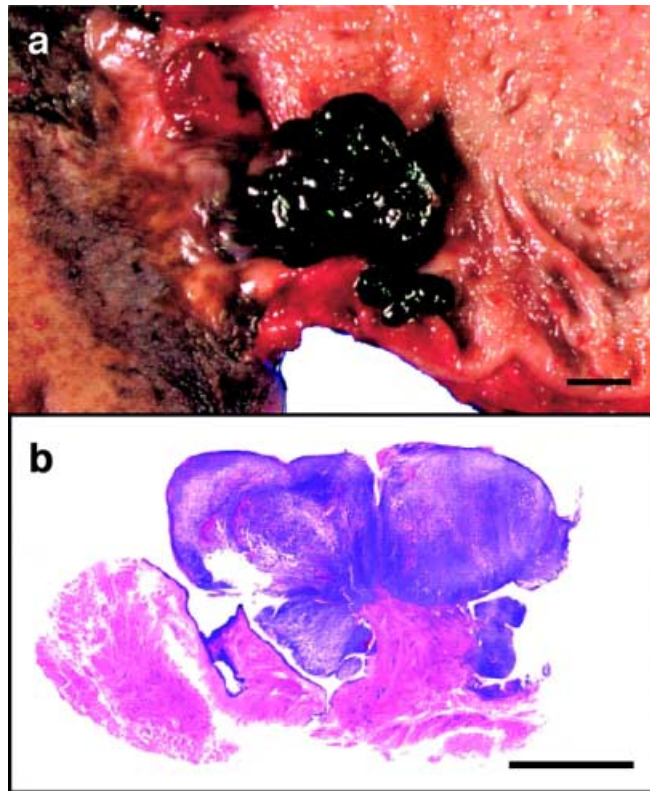


Fig. 2a,b. Malignant melanoma of the anus. **a** A polypoid black tumor is present in the anorectum. **b** The tumor arises on the dentate line, showing fungating growth, and infiltrates to the submucosal layer of the anal canal. Bars, 10mm

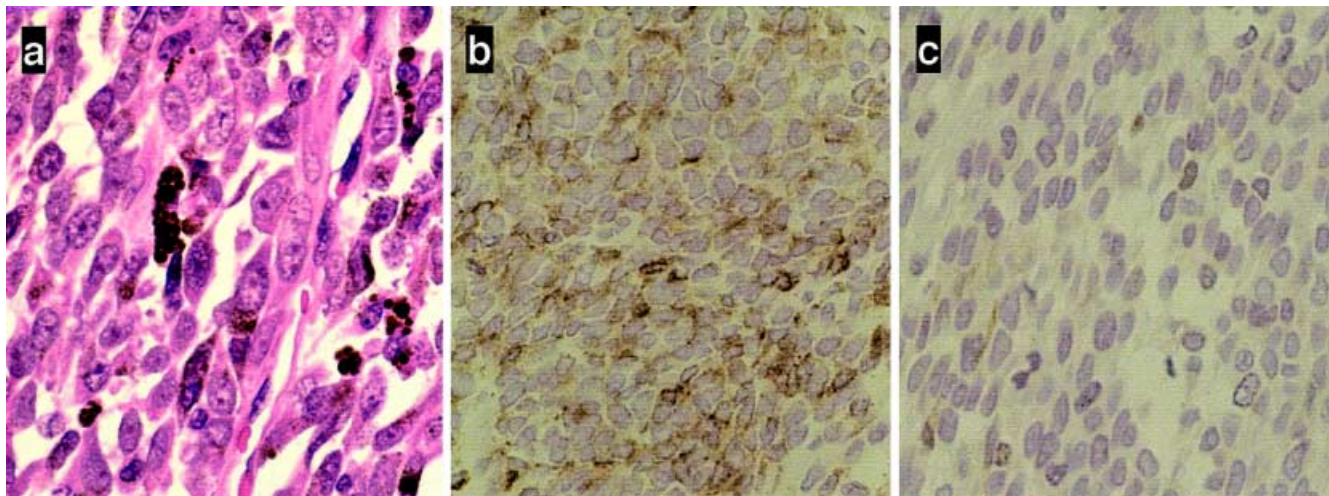


Fig. 3a-c. Histopathology and immunohistochemistry of the anal melanoma. **a** Dense spindle cells with hyperchromatic nuclei, prominent nucleoli, and melanin granules in the cytoplasm. **b** The tumor cells

are positive for HMB45, **c** But almost negative for S100 protein by immunohistochemistry. **a** H&E, $\times 400$; **b** $\times 400$; **c** $\times 400$

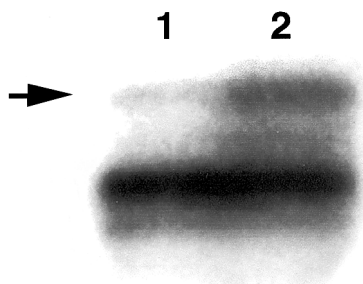


Fig. 4. Allelic loss of the *NF1* gene in the genomic DNA of the anal melanoma. DNAs extracted from the melanoma (*lane 1*) and peripheral blood cells (*lane 2*) were amplified by polymerase chain reaction (PCR) according to the original protocol described by Lázaro et al.²⁵ An allelic loss corresponding to the larger-sized band, indicated by the arrow, is evident

The patient underwent postoperative chemotherapy with dacarbazine, vincristine, and cis-dichlorodiammine platinum. However, multiple liver metastases had arisen soon after the operation, and he died in March 1999.

Discussion

Patients with *NF1* are known to be at high risk of developing certain types of benign and malignant tumors, predominantly those derived from the embryonal neural crest.^{5,6} Because of the marked homology of the *NF1* protein, neurofibromin, to the catalytic domain of GTPase-activating protein, which can downregulate p21^{ras} activity, *NF1* has been suggested to be one of the tumor suppressor genes.⁷⁻¹¹ Losses of heterozygosity of *NF1* with germline or somatic inactivating mutations in one allele have been reported in pheochromocytoma¹⁵ and in neurofibrosarcoma¹³ in patients with *NF1*.

Because *NF1* involves, primarily, tissues derived from the neural crest, and because melanoma arises from normal melanocytes that are of neural crest origin, it has been suggested that *NF1* functions as a tumor suppressor gene in the development of malignant melanoma. Anderson et al.¹² supported this hypothesis; they analyzed eight sporadic malignant melanoma cell lines and found that one had a homozygous deletion of *NF1* and one showed loss of *NF1* expression. Accordingly, it could be assumed that patients with *NF1* would have a high incidence of malignant melanoma. However, only several cases of cutaneous and ocular malignant melanomas in *NF1* have been reported,¹⁶⁻¹⁹ and it is still controversial whether malignant melanoma is more prevalent in patients with *NF1* than in the general population.¹⁶ LOH of the *NF1* gene in malignant melanoma in *NF1* patients has not been described in previous publications.

Malignant melanoma of the anorectum is a rare and usually lethal neoplasia.²⁰⁻²² Anorectal malignant melanomas in *NF1*, in particular, are extremely rare and, to the best of our knowledge, only two case reports have been published to date.^{23,24} The authors of the two previous reports suggested, but did not examine, the involvement of the *NF1*

gene in the carcinogenesis of anorectal malignant melanoma in *NF1*.

In the present patient, LOH of *NF1*, presumably loss of the wild-type allele, was detected in anal malignant melanoma by microsatellite analysis.^{25,28} Inactivation of tumor suppressor genes is often caused by intragenic mutations in one allele, accompanied by deletion of the other wild-type allele. In the tumor reported here, it can be assumed that the retained allele was the mutant *NF1* allele, while the wild-type allele was deleted.

In conclusion, we showed an allelic loss of the *NF1* gene in anal malignant melanoma in a patient with neurofibromatosis type 1. This is the first example which supports the idea that *NF1* can function as a tumor suppressor gene in the development or progression of malignant melanoma in patients with *NF1*.

Acknowledgments We thank Mr. Hiroshu Miura for his technical assistance. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan.

References

- Huson SM, Hughes RAC (1994) The neurofibromatoses: a pathogenetic and clinical overview. Chapman and Hall, London
- Riccardi VM, Eichner JE (1986) Neurofibromatosis: phenotype, natural history and pathogenesis. Johns Hopkins University Press, Baltimore
- Gutmann DH, Aylsworth A, Carey JC, et al. (1997) The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *JAMA* 278:51-57
- National Institutes of Health Consensus Development Conference (1988) Neurofibromatosis: conference statement. *Arch Neurol* 45:575-578
- Bader JL (1986) Neurofibromatosis and cancer. *Ann NY Acad Sci* 486:57-65
- Matsui I, Tanimura M, Kobayashi N, et al. (1993) Neurofibromatosis type 1 and cancer. *Cancer* 72:746-754
- Shen MH, Harper PS, Upadhyaya M (1996) Molecular genetics of neurofibromatosis type 1 (*NF1*). *J Med Genet* 33:2-17
- Viskochil D, Buchberg AM, Xu G, et al. (1990) Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell* 62:187-192
- Wallace MR, Marchuk DA, Anderson LB, et al. (1990) Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three *NF1* patients. *Science* 249:182-186
- Xu G, O'Connell P, Viskochil D, et al. (1990) The neurofibromatosis type 1 gene encodes a protein related to GAP. *Cell* 62:599-608
- Xu G, Tanaka K, Dunn D, et al. (1990) The catalytic domain of the neurofibromatosis type 1 gene product stimulates *ras* GTPase and complements *ira* mutants of *S. cerevisiae*. *Cell* 63:835-841
- Andersen LB, Fountain JW, Gutmann DH, et al. (1993) Mutations in the neurofibromatosis 1 gene in sporadic malignant melanoma cell lines. *Nature Genet* 3:118-121
- Legius E, Marchuk DA, Collins FS, Glover TW (1993) Somatic deletion of the neurofibromatosis type 1 gene in a neurofibrosarcoma supports a tumour suppressor gene hypothesis. *Nature Genet* 3:122-126
- Li Y, Bollag G, Clark R, et al. (1992) Somatic mutations in the neurofibromatosis 1 gene in human tumors. *Cell* 69:275-281
- Xu W, Mulligan LM, Ponder MA, et al. (1992) Loss of *NF1* alleles in pheochromocytomas from patients with type 1 neurofibromatosis. *Genes Chromosom Cancer* 4:337-342

16. Duve S, Rakoski J (1994) Cutaneous melanoma in a patient with neurofibromatosis: a case report and review of the literature. *Br J Dermatol* 131:290–294
17. Specht CS, Smith TW (1988) Uveal malignant melanoma and von Recklinghausen's neurofibromatosis. *Cancer* 62:812–817
18. Stokkel MP, Kroon BB, van der Sande JJ, Neering H (1993) Malignant melanoma associated with neurofibromatosis in two sisters from a family with familial atypical multiple mole melanoma syndrome. *Cancer* 72:2370–2375
19. To KW, Rabinowitz SM, Friedman AH, et al. (1989) Neurofibromatosis and neural crest neoplasms: primary acquired melanosis and malignant melanoma of the conjunctiva. *Surv Ophthalmol* 33:373–379
20. Cooper P, Mills SE, Allen MS Jr (1982) Malignant melanoma of the anus: report of 12 patients and analysis of 255 additional cases. *Dis Colon Rectum* 25:693–703
21. Ross M, Pezzi C, Pezzi T, et al. (1990) Patterns of failure in anorectal melanoma. *Arch Surg* 125:313–316
22. Wanebo HJ, Woodruff JM, Farr GH, Quan SH (1981) Anorectal melanoma. *Cancer* 47:1891–1900
23. Ben-Izhak O, Groisman GM (1995) Anal malignant melanoma and soft-tissue malignant fibrous histiocytoma in neurofibromatosis type 1. *Arch Pathol Lab Med* 119:285–288
24. Garcia Casasola G, Casado A, Ciguenza R, et al. (1992) Melanoma rectal y enfermedad de von Recklinghausen. *Rev Clin Esp* 190:475–476
25. Lázaro C, Gaona A, Xu G, et al. (1993) A highly informative CA/GT repeat polymorphism in intron 38 of the human neurofibromatosis type 1 (*NFI*) gene. *Hum Genet* 92:429–430
26. Kumar R, Smeds J, Lundh Rozell B, Hemminki K (1999) Loss of heterozygosity at chromosome 9p21 (INK4-p14ARF locus): homozygous deletions and mutations in the *p16* and *p14ARF* genes in sporadic primary melanomas. *Melanoma Res* 9:138–147
27. Gyapay G, Morissette J, Vignal A, et al. (1994) The 1993–94 Genethon human genetic linkage map. *Nat Genet* 7(2 Spec No):246–339
28. Cossen MH, van der Est MN, Breuning MH, et al. (1997) Deletions spanning the neurofibromatosis type 1 gene: implications for genotype-phenotype correlations in neurofibromatosis type 1? *Hum Mut* 9:458–464