Jpn. J. Cancer Res. 82, 916–921, August 1991

Chromosome Changes in Desmoid Tumors Developed in Patients with Familial Adenomatous Polyposis

Mitsuaki A. Yoshida,[1,6] Tatsuro Ikeuchi,[1] Takeo Iwama,[2] Michiko Miyaki,[3] Takeo Mori,[4] Yasuhiko Ushijima,[5] Akio Hara,[5] Makoto Miyakita[5] and Akira Tonomura[1]

[1]Department of Cytogenetics, Medical Research Institute and [2]The Center for Analysis of Polyposis and Intestinal Disease, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, [3]Department of Biochemistry, The Tokyo Metropolitan Institute of Medical Science and [4]Department of Surgery, The Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113 and [5]Department of Surgery, National Saitama Hospital, 2-1 Suwa, Wako, Saitama 351-01

Chromosome analyses were performed on benign desmoid tumors obtained from two female patients with familial adenomatous polyposis (FAP), one of whom was diagnosed as having Gardner syndrome (GS). The modal chromosome number was 46 in both specimens, and detailed Q-banding analysis in Case 1 (GS) revealed a clonal abnormality of an interstitial deletion of the long arm of chromosome 5, del(5)(q21q31). The deleted region included an assigned locus for an FAP major gene (5q21-q22). All of the metaphases analyzed in this case showed an extra segment of bright fluorescence on the short arm of chromosome 15, but this unusual chromosome (15p+) was observed in both peripheral lymphocyte and skin fibroblast cultures from the patient, indicating that the 15p+ was constitutional in nature. In Case 2, no clonal rearrangements were identified and most cells had a normal karyotype. However, two cells showed rearrangements involving a 17q with non-identical breakpoints, one of which was observed as a solitary chromosome change. Based on the present findings in Case 1 and those reported so far, the chromosomal defect on 5q might be one of the causal genetic events primarily associated with the development of both benign desmoid tumors and colorectal adenomas and carcinomas in FAP patients.

Key words: Chromosome 5q deletion — Desmoid tumor — Familial adenomatous polyposis — Tumor suppressor gene

Non-random chromosome changes observed in a variety of human malignancies have led to the recognition that they may play a critical role in human tumorigenesis.[1,2] Recent cytogenetic and molecular studies have demonstrated that chromosome deletion or loss at certain genetic loci might be primarily associated with the development of hereditary and sporadic cancers.[3]

Familial adenomatous polyposis (FAP)[7] is a typical cancer-predisposing disease which is inherited as an autosomal dominant trait. Affected persons have numerous adenomatous polyps in the colorectum in early life and, if left untreated, they are at risk of developing colorectal carcinoma. Clinically, some FAP patients show extracolonic manifestations including osteomas and multiple epidermoid cysts, and this category is known as Gardner syndrome.[4] Recent clinical and linkage studies suggest that both FAP and Gardner syndrome may be different types of manifestation resulting from an identical gene mutation.[5,6] Desmoid tumors, known pathologically as benign fibrous tissue tumors, are associated with FAP or Gardner syndrome at higher frequencies than with any other known disease. They develop predominantly in the small bowel mesentery, with some in the abdominal incision or rectum muscle, and also at other sites. The patients affected are predominantly female with a mean age of 30 years.[7,8]

Recently, the major gene responsible for FAP was localized to the region (q21-q22) of chromosome 5,[9–11] and the gene "MCC" located at this region was isolated as a candidate for the putative colorectal tumor suppressor gene.[12] On the other hand, much attention has been focused on the acquired genetic alterations at the chromosomal and molecular levels in colon adenomas and carcinomas developed in FAP patients.[13–22] However, little information is available on the genetic mechanism responsible for benign desmoid tumors, and no chromosomal studies in desmoids from FAP patients have so far been reported.

In the present study, we analyzed the chromosome changes in two desmoid tumors, one from a patient with FAP and the other from a patient with Gardner syndrome, and detected in the latter an interstitial deletion of chromosome 5q, in the region where the FAP gene is located.

[6] To whom correspondence should be addressed: Department of Cytogenetics, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113.

[7] Abbreviations: FAP, familial adenomatous polyposis; LOH, loss of heterozygosity.

CASE REPORTS

Case 1: A 29-year-old woman was diagnosed as having Gardner syndrome with signs of osteoma, odontoma and desmoid tumor. She had undergone surgery for colorectal carcinoma and multiple polyposis, and the desmoid tumor that had developed in the mesentery of the small bowel was excised simultaneously. The excised tumor tissue, weighing approximately 450 g, was subjected to the chromosome study. The recurrence of desmoid has been seen at least 3 times during the subsequent 3 years. We have had no opportunity to study the chromosomes of these tumors.

Case 2: A woman was operated on for multiple colorectal polyposis at the age of 19 years, and the large bowel was partially resected. At 30 years of age, when she was pregnant and admitted to the hospital, desmoid tumors were found at two sites in the abdominal area. These tumors, weighing approximately 70 g and 80 g, respectively, were excised and the former tumor was examined in the present study.

MATERIALS AND METHODS

Fresh tumor specimens obtained directly from surgery were placed in culture medium supplemented with fetal calf serum (FCS) and antibiotics, and transported to our laboratory. The procedures used for enzymatic disaggregation and cell culture of the specimens have been described in our previous reports.[23, 24] In brief, the materials were minced into small pieces with scissors after being washed in Hanks' balanced salt solution (HBSS) containing antibiotics, then digested with 0.8% collagenase (type II) for 1 h at 37°C. The disaggregated cells were washed three times in HBSS and incubated in RPMI1640 medium supplemented with 10% FCS at 37°C in the presence of 5% CO_2.

Chromosome slides were prepared by routine air-drying after treatment with Colcemid (0.02 μg/ml) for 5 h, and karyotype analyses were performed by the Q-staining method.

RESULTS

The cells grown from the primary cultures of the two desmoid tumors showed a fibroblast-like appearance (Fig. 1). They were characterized by a somewhat flattened stellate morphology, large cell size and a cell growth pattern of poorly parallel orientation, in contrast to typical fibroblasts from, for example, skin biopsy samples.

The results of chromosome analysis are summarized in Table I. Mitotic cells suitable for karyotype analysis in Case 1 were obtained from a 5-day culture. A total of 10 Q-banded metaphases were analyzed in detail. Nine of them consistently showed an interstitial deletion on the long arm of chromosome 5, del(5)(q21q31) (Figs. 2 and 3), and in 3 cells in particular, this was the sole chromosome abnormality (Fig. 2). Six cells having the del(5q) showed additional changes in both chromosome structure and number. Another frequent abnormality was loss of the X chromosome, which was identified in 4 metaphases. Although all of the metaphases showed an extra large segment of bright fluorescence on the short arm of chromosome 15 (Fig. 2), this unusual chromosome (15p+) was also identified in all the mitotic cells from both PHA-stimulated lymphocyte cultures (Fig. 4) and cultured skin fibroblasts from the patient, indicating that the 15p+ is constitutional in nature. This type of chromosome, which presumably resulted from translocation of Yq distal segment, can be seen infrequently in the general human population without having phenotypic effects in both males and females.[25] Thus, its presence in the Case 1 patient was regarded as fortuitous. Karyotype analyses on both lymphocyte and skin fibroblast cultures also revealed that the del(5q) present in the desmoid tumor was not constitutional but obviously an acquired abnormality.

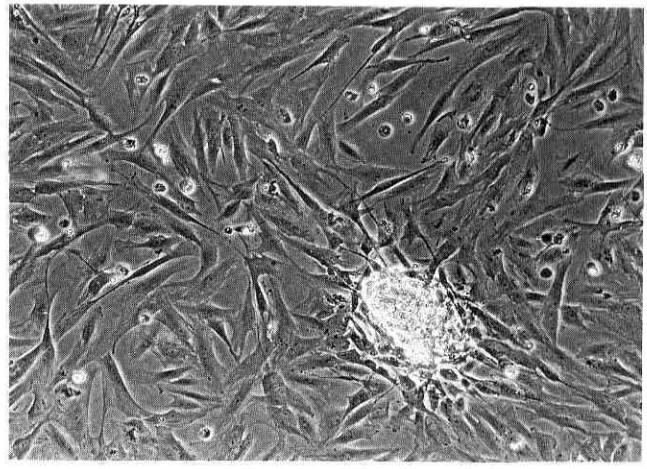

Fig. 1. Cultured fibroblast-like cells grown from the Case 1 desmoid tumor.

In Case 2, a total of 28 mitotic cells were available for karyotype analyses after 58 days of culture. Twenty-four of these showed a normal karyotype and the remaining 4 cells had different structural and/or numerical changes. Rearrangements involving the long arm of chromosome 17 were found in 2 cells, although the breakpoints on 17q were not identical.

Table I. Results of Karyotype Analyses of the Desmoid Tumors

Case No.	No. of cells analyzed	Karyotypes	No. of cells
1	10	46,XX,15p+,del(5)(q21q31)[a]	3
		46,XX,15p+	1
		45,X,15p+,del(5q)	1
		46,X,15p+,del(5q),+12,19p+	1
		46,X,15p+,del(5q),t(14q;?),+marker	1
		47,X,15p+,del(5q),−12,+3 markers	1
		43,XX,15p+,del(5q),−2,−13,−19	1
		42,XX,15p+,del(5q),−2,−8,−12,−22	1
2	28	46,XX	24
		46,XX,t(4;17)(q21;q23)	1
		47,XX,−17,+20,+der(17)t(17;?)(q25;?)	1
		47,XX,+17	1
		46,X,−21,+2 markers	1

a) The del(5)(q21q31) is described in 6 other cells as del(5q), abbreviating the breakpoints.

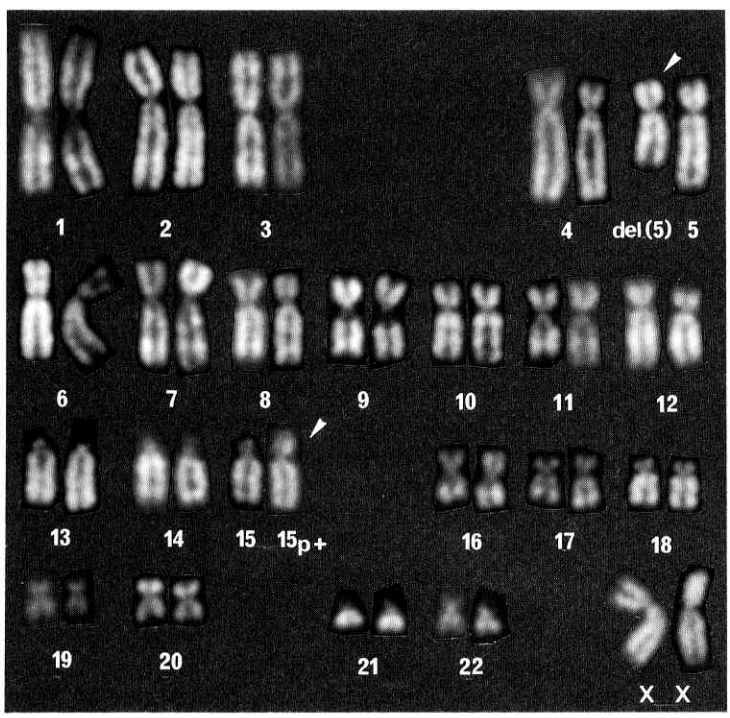

Fig. 2. Q-banded karyotype of the desmoid tumor from Case 1: 46,XX,15p+,del(5)(q21q31). Arrowheads indicate the del(5q) and 15p+ chromosomes (see text).

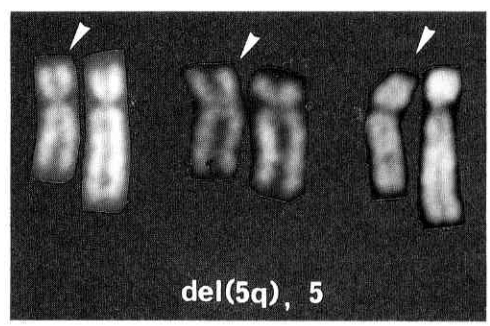

Fig. 3. Q-banded chromosome #5 pairs from 3 different desmoid tumor cells in Case 1, showing the del(5q) chromosomes.

DISCUSSION

Chromosome analyses were carried out successfully on two desmoid tumors that had developed in female patients diagnosed as having FAP and Gardner syndrome, respectively. Of special interest was the finding that an interstitial deletion on the long arm of chromosome 5, del (5q), was present as a clonal change in one of the two desmoid tumors, and that in some cells the del(5q) was the sole chromosome abnormality. Since the deleted segment of 5q21-q31 involves the assigned locus of the FAP gene (5q21-q22),[26] the above findings suggest that the del(5q) might have been a primary chromosomal alteration responsible for the development of the desmoid tumor.

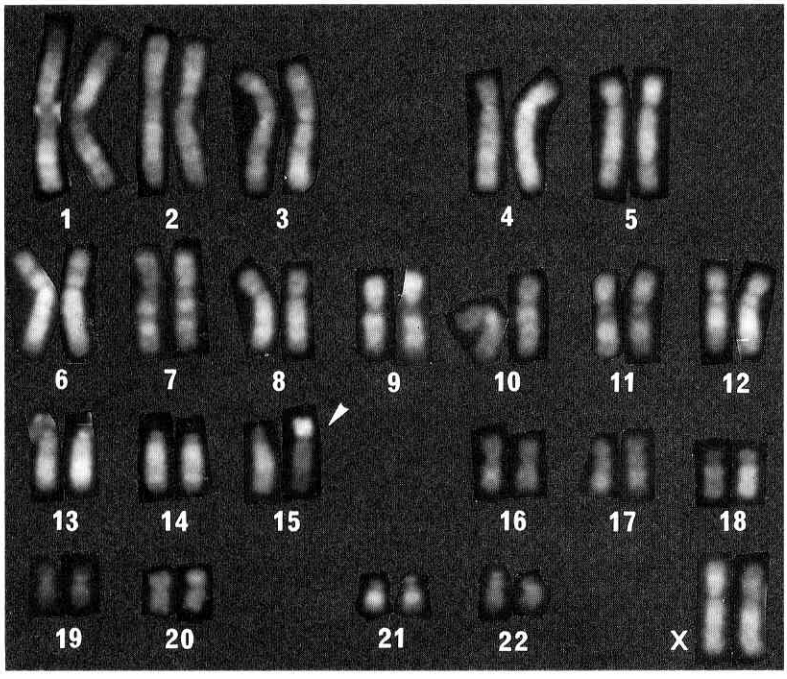

Fig. 4 Q-banded karyotype of a PHA-stimulated cultured blood lymphocyte from Case 1. The arrowhead indicates the 15p+ chromosome identical to that seen in Fig. 2.

The del(5q) finding in Case 1 was in good agreement with molecular analyses by Okamoto *et al.*,[27] who showed a loss of heterozygosity (LOH) in this tumor (corresponding to their case no. PLK42-D) using a D5S 81 DNA probe. Thus, the present study proved that the LOH on 5q was attributable to an interstitial chromosome deletion of 5q. The above authors also demonstrated by haplotype analysis of the tumor and family members that the allele lost was from the unaffected father, while the affected mother's allele was retained in the tumor. This means that the chromosome involved in the deletion was one bearing a normal wild-type FAP gene, and that the normal chromosome 5 possessed the mutant gene transmitted from the affected mother. This finding is consistent with the assumption that the loss of function of both homologous FAP genes, by analogy to retinoblastoma and Wilms' tumors, would be primarily associated with the development of this tumor.

LOH on 5q has been reported in both adenomatous polyps and colon carcinomas from sporadic and inherited cases, with a progressive increase in freguency from mild to severe dysplasia to advanced carcinoma.[15, 18, 19, 22] Deletion of chromosomal material on 5q has also been frequently observed in adenomas from FAP patients.[21] Recent molecular and chromosomal studies of colon carcinomas and adenomas, occurring genetically or sporadically, have indicated frequent allelic and chromosomal losses on chromosomes other than 5q, including 17p, 18q and 22q.[13-22] The incidences of these losses were also higher in carcinomas and adenomas with high-grade dysplasia.[15, 18, 19, 22] This series of studies described above has led to the proposal that accumulated losses of multiple tumor suppressor genes are significantly associated with the conversion of normal cells into fully malignant cells.

Presumably, the acquired genetic alterations in benign desmoid tumors would be rather mild in degree, as in adenomatous polyps, compared with malignant carcinomas. In the desmoid tumors presented here, karyotypic alterations were not so extensive: the tumor in Case 1 showed no clonal chromosome abnormalities other than del(5q), and in the tumors of Case 2 most cells were karyotypically normal. Although the fibroblasts grown in primary cultures from the desmoid tumors showed an atypical morphology, the possibility still exists that the cells with a normal karyotype in Case 2 might have been normal fibroblasts indistinguishable from the unusually proliferating fibroblasts responsible for the genesis of desmoids. However, a fraction of the cells in Case 2 showed some numerical and/or structural chromosome abnormalities, suggesting that at least these cells were of tumor origin.

The present study is the first to report chromosome abnormalities in desmoid tumors from FAP patients. One desmoid tumor in a sporadic case has been chromosomally studied,[28] and 11 clonal markers involving chromosomes 1, 3, 6, 10, 12, 21 and 22 were identified. However, there were no abnormalities common to those seen in the present two tumor specimens.

At present, it seems likely that the FAP gene mutation alone is not sufficient for the hyperproliferation of fibrous tissue cells, resulting in enlarged desmoid tumors, and that certain acquired genetic alterations including chromosome mutations such as del(5q) are necessary. Further combined studies on both chromosomal and molecular bases will be needed to achieve a better understanding of the genetic mechanisms responsible for the genesis of desmoid and extracolonic tumors in FAP patients. Investigations along these lines would also help to clarify the intriguing question of why the desmoid and other extracolonic tumors from FAP patients hardly show malignant conversion.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan.

(Received April 3, 1991/Accepted June 6, 1991)

REFERENCES

1) Sandberg, A. A. A chromosome hypothesis of oncogenesis. *Cancer Genet. Cytogenet.*, **81**, 277–285 (1983).
2) Mitelman, F. "Catalog of Chromosome Aberrations in Cancer," 2nd Ed. (1985). Alan R. Liss, New York.
3) Knudson, A. G. Hereditary cancer, oncogenes and antioncogenes. *Cancer Res.*, **45**, 1437–1443 (1985).
4) Gardner, E. J. Follow-up study of a family group exhibiting dominant inheritance for a syndrome including intestinal polyps, osteomas, fibromas and epidermal cysts. *Am. J. Hum. Genet.*, **14**, 376–390 (1962).
5) Nakamura, Y., Lathrop, M., Leppert, M., Dobbs, M., Wasmuth, J., Wolff, E., Carlson, M., Fujimoto, E., Krapcho, K., Sears, T., Woodward, S., Hughes, J., Burt, R., Gardner, E., Lalouel, J. M. and White, R. Localization of the genetic defect in familial adenomatous polyposis within a small region of chromosome 5. *Am. J. Hum. Genet.*, **43**, 638–644 (1988).
6) Utsunomiya, J. Pathology, genesis and management of hereditary gastrointestinal polyposes. *In* "Genetic Epidemiology of Cancer," ed. H. T. Lynch and T. Hirayama, pp. 219–249 (1989). CRC Press, Inc., Boca Raton, Florida.
7) Jagelman, D. G. Extracolonic manifestations of familial polyposis coli. *Cancer Genet. Cytogenet.*, **27**, 319–325 (1987).
8) Klemmer, S., Pascoe, L. and De Cosse, J. Occurrence of desmoids in patients with familial adenomatous polyposis of the colon. *Am. J. Hum. Genet.*, **28**, 385–392 (1987).
9) Herrera, L., Kakati, S., Gibas, L., Pietrzak, E. and Sandberg, A. A. Brief clinical report: Gardner syndrome in a man with an interstitial deletion of 5q. *Am. J. Med. Genet.*, **25**, 473–476 (1986).
10) Bodmer, W. F., Bailey, C. J., Bodmer, J., Bussey, H. J. R., Ellis, A., Gorman, P., Lucibello, F. C., Murday, V. A., Rider, S. H., Scambler, P., Sheer, D., Solomon, E. and Spurr, N. K. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature*, **328**, 614–616 (1987).
11) Leppert, M., Dobbs, M., Scambler, P., O'Connell, P., Nakamura, Y., Stauffer, D., Woodcoard, S., Burt, R., Hughes, J., Gardner, E., Lathrop, M., Wasmuth, J., Lalouel, J. M. and White, R. The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science*, **241**, 1411–1414 (1987).
12) Kinzler, K. W., Nilbert, M. C., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hamilton, S. R., Hedge, P., Markham, A., Carlson, M., Joslyn, G., Groden, J., White, R., Miki, Y., Miyoshi, Y., Nishisho, I. and Nakamura, Y. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science*, **251**, 1366–1370 (1991).
13) Muleris, M., Salmon, R. J., Zafrani, B., Girodet, J. and Dutrillaux, B. Consistent deficiencies of chromosome 18 and of the short arm of chromosome 17 in eleven cases of human large bowl cancer: a possible recessive determinism. *Ann. Génét.*, **28**, 206–213 (1985).
14) Solomon, E., Voss, R., Hall, V., Bodmer, W. F., Jass, J. R., Jeffreys, A. J., Lucibello, F. C., Patel, I. and Rider, S. H. Chromosome 5 allele loss in human colorectal carcinomas. *Nature*, **328**, 616–619 (1987).
15) Okamoto, M., Sasaki, M., Sugio, K., Sato, C., Iwama, T., Ikeuchi, T., Tonomura, A., Sasazuki, T. and Miyaki, M. Loss of constitutional heterozygosity in colon carcinoma from patients with familial polyposis coli. *Nature*, **331**, 273–277 (1988).
16) Monpezat, J-P., Delattre, O., Bernard, A., Grunwald, D., Remvikos, Y., Muleris, M., Salmon, R. J., Frelat, G., Dutrillaux, B. and Thomas, G. Loss of alleles on chromosome 18 and on the short arm of chromosome 17 in polyploid colorectal carcinomas. *Int. J. Cancer*, **41**, 404–408 (1988).
17) Law, D. J., Olschwang, S., Monpezat, J-P., Lefrancois, D., Jagelman, D., Petrelli, N. J., Thomas, G. and Feinberg, A. P. Concerted nonsyntenic allelic loss in human colorectal carcinoma. *Science*, **241**, 961–965 (1988).
18) Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M. and Bos, J. L. Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.*, **319**, 525–532 (1988).
19) Sasaki, M., Okamoto, M., Sato, C., Sugio, K., Soejima, J., Iwama, T., Ikeuchi, T., Tonomura, A., Miyaki, M. and

Sasazuki, T. Loss of constitutional heterozygosity in colorectal tumors from patients with familial polyposis coli and those with nonpolyposis colorectal carcinoma. *Cancer Res.*, **49**, 4402–4406 (1989).
20) Ashton-Rickardt, P. G., Dunlop, M. G., Nakamura, Y., Morris, R. G., Purrdic, C. A., Sted, C. M., Evans, H. J., Bird, C. C. and Wyllie, A. H. High frequency of APC loss in sporadic colorectal carcinoma due to breaks clustered in 5q21-22. *Oncogene*, **4**, 1169–1174 (1989).
21) Ikeuchi, T., Yoshida, M. A., Iwama, T., Miyaki, M., Okayasu, I. and Tonomura, A. Cytogenetic studies on colon carcinomas and adenomas from patients with familial polyposis coli. *In* "Hereditary Colorectal Cancer," ed. J. Utsunomiya and H. T. Lynch, pp. 533–540 (1990). Springer-Verlag, Tokyo.
22) Miyaki, M., Seki, M., Okamoto, M., Yamanaka, A., Maeda, Y., Tanaka, K., Kikuchi, R., Iwama, T., Ikeuchi, T., Tonomura, A., Nakamura, Y., White, R., Miki, Y., Utsunomiya, J. and Koike, M. Genetic changes and histopathological types in colorectal tumors from patients with familial adenomatous ploypsis. *Cancer Res.*, **50**, 7166–7173 (1990).
23) Yoshida, M. A., Ohyashiki, K., Ochi, H., Gibas, Z., Pontes, J. E., Huben, G. R. and Sandberg, A. A. Cytogenetic studies of tumor tissue from patients with nonfamilial renal cell carcinoma. *Cancer Res.*, **46**, 2139–2147 (1986).
24) Yoshida, M. A., Ikeuchi, T., Tachibana, Y., Takagi, K., Moriyama, M. and Tonomura, A. Rearrangements of chromosome 3 in nonfamilial renal cell carcinomas from Japanese patients. *Jpn. J. Cancer Res.*, **79**, 600–607 (1988).
25) Nielsen, J. and Rasmussen, K. Y/autosomal translocations. *Clin. Genet.*, **9**, 609–617 (1976).
26) Human gene mapping 10.5(1990): update to the Tenth International Workshop on Human Gene Mapping. *Cytogenet. Cell Genet.*, **55**, 1–785 (1990).
27) Okamoto, M., Sato, C., Kohno, Y., Mori, T., Iwama, T., Tonomura, A., Miki, Y., Utsunomiya, J., Nakamura, Y., White, R. and Miyaki, M. Molecular nature of chromosome 5q loss in colorectal tumors and desmoids from patients with familial adenomatous polyposis. *Hum. Genet.*, **85**, 595–599 (1990).
28) Karlsson, I., Mandahl, N., Heim, S., Rydholm, A., Willen, H. and Mitelman, F. Complex chromosome rearrangements in an extraabdominal desmoid tumor. *Cancer Genet. Cytogenet.*, **34**, 241–245 (1988).