

Brief Report

GENETIC ANALYSIS OF A SARCOMA ACCIDENTALLY TRANSPLANTED FROM A PATIENT TO A SURGEON

HERMINE-VALERIA GÄRTNER, M.D., CHRISTIAN SEIDL, M.D.,
CHRISTINE LUCKENBACH, PH.D., GEORG SCHUMM, M.D.,
ERHARD SEIFRIED, M.D., HORST RITTER, M.D.,
AND BURKHARD BÜLTMANN, M.D.

MODERN concepts of cancer immunology originated from the classic observations by Jensen, Loeb, Tyzzer, and Little in the early years of the 20th century of the rejection of transplanted allogeneic tumors and the acceptance of syngeneic tumors.¹ Despite this law of transplantation, there are several clinical examples of the accidental transplantation of a malignant tumor or tumor cells into a healthy recipient.²⁻⁵

We describe the accidental transplantation of a malignant sarcoma from a patient to a surgeon. Using molecular methods, we showed that the sarcomas in the unrelated patient and surgeon were genetically identical.

CASE REPORT

A 32-year-old man underwent emergency surgery to remove a malignant fibrous histiocytoma from his abdomen and died shortly thereafter of postoperative complications. During the operation the 53-year-old surgeon injured the palm of his left hand while placing a drain. The lesion was immediately disinfected and dressed. Five months later, the surgeon consulted a hand specialist because of a hard, circumscribed, tumor-like swelling, 3.0 cm (1.2 in.) in diameter, in his left palm at the base of the middle finger, where he had been injured during the operation. An extensive examination, including laboratory tests, did not reveal any signs of immune deficiency. The tumor was completely excised. Histologic examination revealed that it was a malignant fibrous histiocytoma. Two years later, the surgeon's condition was good, and there was no evidence of recurrence or metastasis of the tumor.

The pathologist who investigated both the patient's tumor and the surgeon's tumor raised the question whether the tumors were identical.

METHODS

Histologic and Immunohistologic Analysis

Samples of tumor tissue from the patient and surgeon were embedded in paraffin and stained with hematoxylin and eosin, peri-

odic acid–Schiff, and van Gieson's stain. Immunostaining was performed with the avidin–biotin–peroxidase method, with antibodies against vimentin, lysozyme, alpha₁-antitrypsin, alpha₁-antichymotrypsin, keratin, endomysial antigens, S100, actin, and desmin (all antibodies were obtained from Dako, Glostrup, Denmark).

Isolation of DNA

Genomic DNA from peripheral-blood samples was isolated by the "salting-out" method.⁶ DNA from paraffin-embedded tumor and tissue samples was extracted according to a modification⁷ of the method of Goelz et al.⁸

Analysis of Short Tandem-Repeat Polymorphisms

Short tandem-repeat polymorphisms of the loci HUMTH01, HUMCYAR04, and HUMACTBP2 were amplified by the polymerase chain reaction (PCR) with fluorescence-labeled primers. Primer sequences were chosen from published sequences.⁹⁻¹¹ The 5' primer for HUMCYAR04 was labeled with 5-carboxylfluorescein, whereas the 5' primers for HUMTH01 and HUMACTBP2 were labeled with 6-carboxy-2',4',7',4',7-hexachlorofluorescein. PCR products were analyzed with an automated DNA sequencer (model 373A, Applied Biosystem Division, Perkin-Elmer, Foster City, Calif.).

Sequence-Based Typing of HLA Genes

Typing of *HLA-DRB1* and *DQB1* alleles was performed by allele-specific PCR amplification in combination with solid-phase direct DNA sequencing. Sequence analysis was performed on an automated DNA sequencer (model 373A, Perkin-Elmer). Primer sequences were chosen from genomic sequences of *HLA-DRB1* and *DQB1*¹² or from published sequences.¹³

RESULTS

Histologic analysis of tumor tissues from the surgeon and the patient revealed that they were morphologically identical. Both tumors were malignant fibrous histiocytomas of the storiform–pleomorphic subtype (Fig. 1A). They consisted mainly of fibroblast-like and histiocyte-like cells, arranged in a fascicular and storiform pattern, intermingled with some pleomorphic cells and a few inflammatory cells. There were numerous mitotic figures and many necrotic areas. In the periphery of the surgeon's tumor, there was intense inflammation, with an infiltrate consisting mainly of lymphocytes and macrophages and few plasma cells (Fig. 1B). Both tumors stained for vimentin, alpha₁-antitrypsin, and alpha₁-antichymotrypsin.

Analysis of short tandem-repeat sequences clearly demonstrated a chimeric constellation of alleles in the surgeon's tumor (Fig. 1C). Allele 11 (187 bp) of HUMCYAR04, allele 8 (166 bp) of HUMTH01, and allele 31 (300 bp) of HUMACTBP2 were detected in the tumors from both the patient and the surgeon (Table 1). To rule out a tumor-specific genetic pattern of these short tandem-repeat polymorphisms, a DNA sample from another malignant fibrous histiocytoma, histologically identical to the tumors of the patient and the surgeon, was analyzed. The allelic profile of this control malignant fibrous histiocytoma, identified by analysis of short tandem-repeat sequences, was clearly distinct from that of the patient's and the surgeon's tumors (Table 1).

Sequence analysis of *HLA-DRB1* and *DQB1* genes revealed a constellation of heterozygous alleles in the

From the Institutes of Pathology (H.-V.G., B.B.) and Anthropology and Human Genetics (C.L., H.R.), Eberhard-Karls-Universität, Tübingen; the Institute of Transfusion Medicine and Immunohematology, Red Cross Blood Donor Service, Frankfurt am Main (C.S., E.S.); and the Institute of Pathology, General Hospital, Heilbronn (G.S.) — all in Germany. Address reprint requests to Dr. Gärtner at the Institute of Pathology, Eberhard-Karls-Universität, Liebermeisterstraße 8, D-72076 Tübingen, Germany.

©1996, Massachusetts Medical Society.

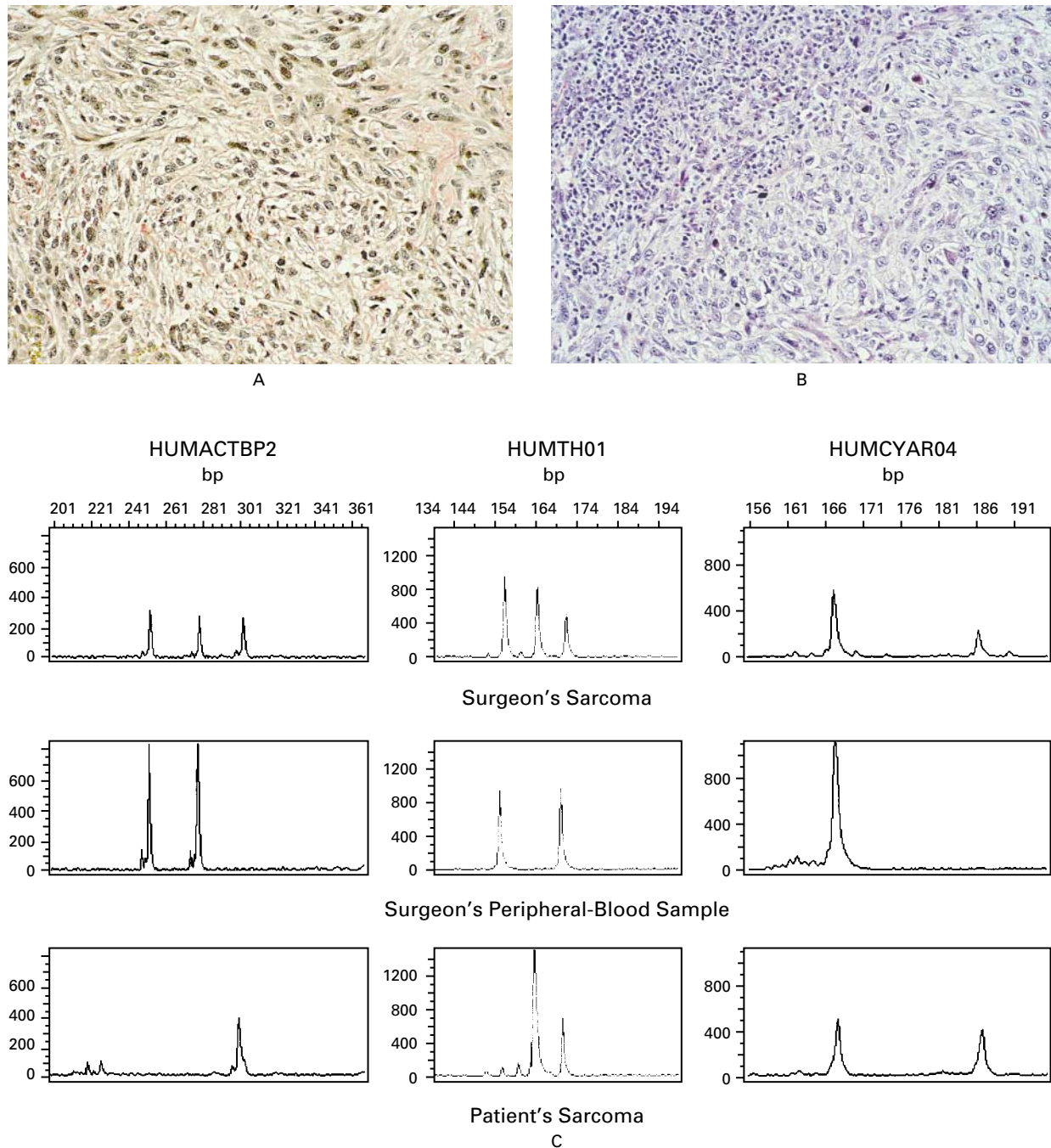


Figure 1. Histologic Findings (Panels A and B) and Immunologic Findings (Panel C) on Analysis of the Patient's and the Surgeon's Tumors.

Both the patient's tumor (Panel A; van Gieson's stain, $\times 20$) and the surgeon's tumor (Panel B; hematoxylin and eosin, $\times 20$) were malignant fibrous histiocytomas. The surgeon's tumor was surrounded by an inflammatory process, with dense infiltrates, consisting mainly of lymphocytes and macrophages. Panel C shows electrophoretograms of short tandem-repeat sequences of loci HUMACTBP2, HUMTH01, and HUMCYAR04. Peaks represent fluorescence intensities of dye-labeled DNA products.

TABLE 1. RESULTS OF ANALYSIS OF POLYMORPHIC SHORT TANDEM-REPEAT SEQUENCES AND HLA ANALYSIS.*

LOCUS	PATIENT'S TUMOR	SURGEON'S TUMOR	SURGEON'S PERIPHERAL-BLOOD SAMPLE	CONTROL TUMOR
	allele (bp)			
HUMCYAR04	7-3 (168)† 11 (187)	7-3 (168)† 11 (187)	7-3 (168)†	11 (187)
HUMTH01	8 (166) 10 (173)	6 (158) 10 (173) 8 (166)	6 (158) 10 (173)	6 (158) 9 (170)
HUMACTBP2	31 (300) 31 (300)	19 (252) 25 (277) 31 (300)	19 (252) 25 (277)	21 (258) 28 (289)
HLA-DRB1	1501 1401	01 07 1501 1401	01 07	ND
HLA-DQB1	05031 0602	0501 02 05031 0602	0501 02	ND

*The results were obtained by computer analysis (Genescan software, Perkin-Elmer). ND denotes not done.

†Allele 7-3 contains a deletion of 3 bp in the 5' flanking region of the repeat sequence.

patient's tumor and in the peripheral blood of the surgeon (Table 1). All four alleles, two from the patient's tumor and two from the surgeon's blood cells, were present in the tumor sample from the surgeon.

DISCUSSION

We used histologic and immunohistologic methods, analysis of short tandem-repeat polymorphisms, and sequence-based typing of HLA genes to determine the genetic origin of a sarcoma that had been accidentally transplanted from a patient to a surgeon. Both the pattern of short tandem-repeat sequences and the chimeric constellation of HLA alleles in the surgeon's tumor identified the genetic origin of the sarcoma. The patient and the surgeon had different HLA haplotypes, with complete discrepancies of *DRB1* and *DQB1* alleles. The patient died before we could perform HLA class I typing, but from the linkage disequilibrium between HLA genes we can assume that there were also major class I mismatches between the patient and the surgeon.

Normally, transplantation of allogeneic tissue from one person to another induces an immune response that leads to the rejection of the transplanted tissue.^{1,14-17} In the case of the surgeon, an intense inflammatory reaction developed in the tissue surrounding the tumor, but the tumor mass increased, suggesting an ineffective antitumor immune response. The tumor may have escaped immunologic destruction through several mechanisms, such as qualitative

and quantitative changes of major histocompatibility complex class I molecules on the tumor cells, an absence of immunogenic tumor antigens,¹⁸⁻²² deficient antigen processing by the tumor, or deficient presentation of tumor antigens by the host's antigen-presenting cells in the absence of costimulatory signals with consequent T-cell and B-cell anergy.²³⁻²⁵

We are indebted to Dr. Hans Georg Rammensee for his critical review of the manuscript and to Utz Bacher for his excellent secretarial assistance.

REFERENCES

- Klein J. Natural history of the major histocompatibility complex. New York: John Wiley, 1986.
- Southam CM. Homotransplantation of human cell lines. *Bull NY Acad Med* 1958;34:416-23.
- Nadler SH, Moore GE. Immunotherapy of malignant disease. *Arch Surg* 1969;99:376-81.
- Scanlon EF, Hawkins RA, Fox WW, Smith WS. Fatal homotransplanted melanoma: a case report. *Cancer* 1965;18:782-9.
- Gugel EA, Sanders ME. Needle-stick transmission of human colonic adenocarcinoma. *N Engl J Med* 1986;315:1487.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215-8.
- Luckenbach C, Gärtner HV, Seidl C, Ritter H. Tumor inoculation between two unrelated human individuals: STR analysis of paraffin-embedded tissue section. *Adv Forensic Haemogenet* 1996;6:192-4.
- Goelz SE, Hamilton SR, Vogelstein B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem Biophys Res Commun* 1985;130:118-26.
- Polymeropoulos MH, Xiao H, Rath DS, Merrill CR. Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH). *Nucleic Acids Res* 1991;19:3753.
- Idem*. Tetranucleotide repeat polymorphism at the human aromatase cytochrome P-450 gene (CYP19). *Nucleic Acids Res* 1991;19:195.
- Idem*. Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2). *Nucleic Acids Res* 1992;20:1432.
- Marsh SGE, Bodmer JG. HLA class II nucleotide sequences, 1992. *Tissue Antigens* 1992;40:229-43.
- Bein G, Gläser R, Kirchner H. Rapid HLA-DRB1 genotyping by nested PCR amplification. *Tissue Antigens* 1992;39:68-73.
- Boon T. Toward a genetic analysis of tumor rejection antigens. *Adv Cancer Res* 1992;58:177-210.
- Moller P, Hammerling GJ. The role of surface HLA-A,B,C molecules in tumor immunity. *Cancer Surv* 1992;13:101-27.
- Schreiber H. Tumor immunology. In: Paul WE, ed. *Fundamental immunology*. 3rd ed. New York: Raven Press, 1993:1143-78.
- Colonna M. Natural killer cell receptors specific for MHC class I molecules. *Curr Opin Immunol* 1996;8:101-7.
- Goodenow RS, Vogel JM, Linsk RL. Histocompatibility antigens on murine tumors. *Science* 1985;230:777-83.
- Tanaka K, Yoshioka T, Bieberich C, Jay G. Role of the major histocompatibility complex class I antigens in tumor growth and metastasis. *Annu Rev Immunol* 1988;6:359-80.
- Ljunggren HG, Kärre K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 1990;11:237-44.
- Travers PJ, Arklie JL, Trowsdale J, Patillo RA, Bodmer WF. Lack of expression of HLA-ABC antigens in choriocarcinoma and other human tumor cell lines. In: Greenwald P, ed. *Research frontiers in aging and cancer: international symposium for the 1980's*. NCI monograph 60. Washington, D.C.: Government Printing Office, 1982:175-80. (NIH publication no. 82-2436).
- Rammensee HG, Fischer Lindhal K. Less scholasticism, more exact immunology. *Curr Opin Immunol* 1996;8:49-50.
- Suthanthiran M. Transmembrane signaling requirements of T-cells: implications for regulation of alloimmunity. *Transplant Proc* 1995;27:Suppl 1:5-7.
- Boussiotis VA, Gribben JG, Freeman GJ, Nadler LM. Blockade of the CD28 co-stimulatory pathway: a means to induce tolerance. *Curr Opin Immunol* 1994;6:797-807.
- McHugh RS, Ahmed SN, Wang YC, Sell KW, Selvaraj P. Construction, purification and functional incorporation on tumor cells of glycolipid-anchored human B7-1 (CD80). *Proc Natl Acad Sci U S A* 1995;92:8059-63.