Transpl Int (1998) 11: 382–386

BRIEF REPORT

Cornelia Schmitt
Kirsten Ciré
Stephanie Schattenkirchner
Manfred Pollok
Claus A. Baldamus
Barbara Krug
Thomas Kirchner
Peter H. Wünsch
Thomas Krieg
Michael Staak

Highly sensitive DNA typing for detecting tumors transmitted by transplantation

Received: 25 July 1997
Received after revision: 27 March 1998
Accepted: 18 May 1998

C. Schmitt (✉) · M. Staak
Institute of Forensic Medicine,
University of Cologne,
Melatengürtel 60–62,
D-50823 Köln, Germany
Fax: + 49 221 478 4261

K. Ciré · S. Schattenkirchner · T. Krieg
Department of Dermatology,
University of Cologne,
D-50823 Köln, Germany

M. Pollok · C. A. Baldamus
Department of Internal Medicine,
University of Cologne,
D-50823 Köln, Germany

B. Krug
Institute of Radiology,
University of Cologne,
D-50823 Köln, Germany

T. Kirchner · P. H. Wünsch
Institute of Pathology,
University of Erlangen-Nürnberg,
Nürnberg, Germany

Abstract DNA typing of a variable number of tandem repeats (VNTRs) and of short tandem repeats (STRs) is a modern forensic method for the identification of biological material. In many cases, amplification by the polymerase chain reaction (PCR), especially of STRs, allows DNA typing of minute amounts of or degraded DNA. Here we describe the successful use of forensic DNA typing to clarify the origin of a malignant tumor. We report two cases of metastatic malignant melanoma of unknown origin that developed a few months after transplantation in two recipients of kidneys from the same donor. Fresh metastatic tissue and blood from the first recipient, reference DNA of the donor, and only paraffin-embedded tissue from the second recipient were available for analysis. To investigate whether the melanoma originated in the donor, DNA analysis of nine polymorphic loci was performed. The results of the analysis showed that, in both cases, the tumors were genetically different from the recipient DNA but matched the donor DNA. One incident of allele loss was attributed to a mutation event. We conclude that the metastatic melanoma in both recipients originated in the donor and was transmitted by renal transplantation.

Key words Forensic DNA typing · Short tandem repeat · Tumor transmission · Renal transplantation · Malignant melanoma

Introduction

Organ transplant recipients are at high risk of developing malignancies due to immunosuppressive therapies. Although the cases reported in the literature are rare, the problem demands increasing attention due to the frequency of organ transplantation in recent years. In addition, transplant recipients risk accidental transmission of malignant cells from clinically healthy donors [15]. This often raises the question of where the malignant tumors originated.

DNA typing of a variable number of tandem repeats (VNTR) [4, 11, 18], and especially short tandem repeats (STR) [8], is now the first choice for forensic analysis, i.e., for identification of biological stains and paternity testing. DNA typing is widely accepted by both criminal investigators and lawyers in Europe. Because of their low molecular length (80–400 bp), STRs can be ana-

lyzed using the polymerase chain reaction (PCR). This allows genetic typing of even minute amounts or highly degraded DNA [5, 19], e.g., DNA extracted from paraffin-embedded tissue or histological slides. This approach is not only sensitive, but a combination of several STRs has an extremely high power of discrimination and is quite likely to be able to differentiate between donor and recipient genotypes. This individualization might not be possible with HLA typing, especially since the donor and recipient HLA-geno-type may match. If the amount of material is sufficient for HLA typing, STRs may provide an additional validation of the HLA identification results.

Here we present two cases in which two patients developed post-transplant metastasis of malignant melanomas that could be traced back to the clinically healthy donor. We demonstrate that forensic DNA typing is a useful method to identify the origin of a malignant tumor that could help in legal proceedings [3].

Case reports

Case 1

A 46-year-old female patient received a kidney allograft from a 73-year-old female donor who died of cerebral hemorrhage without any other abnormalities. Nine months after transplantation, the recipient developed pigmented and nonpigmented subcutaneous nodules. Histopathological examination of a skin biopsy revealed a metastasis of a malignant melanoma. There was no obvious primary site. A few weeks later, the patient died of metastatic malignant melanoma.

Case 2

A 43-year-old female recipient of a renal transplant from the same donor developed histopathologically confirmed metastases of a malignant melanoma 7 months after transplantation. The primary site was unknown. Four months later, the recipient died of metastatic malignant melanoma.

Materials and methods

To investigate the origin of the recipients' tumors, nine independent, polymorphic DNA loci were amplified using PCR. DNA from fresh metastatic tissue and blood from the first recipient, as well as from metastasis and tumor-free, paraffin-embedded tissue from the second recipient was isolated. DNA (spleen) from the donor was kindly provided by Eurotransplant (Leiden, the Netherlands).

DNA was extracted after proteinase K lysis [9] with phenol chloroform and concentrated with Centricon 100 (Amicon). The following loci were amplified: HumVWA [12], HumTHO1 [8], HumFES [16], HumFGA [14], HumACTBP2 [17], D3S1358 [13], D21S11 [20], D1S80 [11], and Apo B [4]. Primers were synthesized and labelled by Pharmacia Biotech Freiburg (desalting by NAP-10 column) as described by the authors, except for the primers for D3S1358, which were from the AmpFlSTR Blue kit (Perkin Elmer/ABD, Foster City, Calif., USA). Amplification of the STR systems VWA, THO1, FES, FGA, ACTBP2, and D21S11 was carried out in 25 µl using the following parameters: 1 U Taq polymerase (Promega, Madison, Wis., USA), 0.25 µM each primer (FGA, ACTBP2, D21S11: 5'labelled with fluorescein), 150 µM dNTPs, 10 mM TRIS-HCl pH 8.3, 1.5 mM $MgCl_2$, and 0.1 % Triton X-100 in 25 µl. The temperature cycles were: 94 °C for 1 min., 54 °–61 °C (depending on the loci) for 1 min, 72 °C for 2 min; 28–31 cycles. The amplification of the VNTR systems Apo B and D1S80 in 50 µl was: 2U Taq polymerase (Promega), 0.5 µM each primer, 150 µM dNTPs, 10 mM TRIS-HCl pH 8.3, 1.5 mM $MgCl_2$, and 0.1 % Triton X-100 in 50 µl. The temperature cycles were: 94 °C for 1 min, 58 °C for 1 min, 72 °C for 4 min (Apo B), and 94 °C for 1 min, 63 °C for 1 min, and 72 °C for 8 min (D1S80); 29 cycles (Apo B) and 27 cycles (D1S80). The amplification of D3S1358 (labelled 5'FAM primer) was performed in 25 µl according to the manufacturer's recommendation using 2.5 U AmpliTaq Gold. The temperature cycles were: 95 °C for 11 min, followed by 29 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min. A final extension was conducted at 60 °C for 30 min. All amplifications were carried out on a Trio Thermoblock (Biometra, Germany).

The PCR products were separated on 20-cm nondenaturing polyacrylamide gels (Apo B 5 % T 3 % C, D1S80 6 % T 3 % C, VWA, TH01, FES 7.2 % T 3 % C), crosslinked with piperazine diacrylamide, cast on Gelbond PAG film (FMC, Rockland, Me., USA by Biozym, Germany). The gels were 0.75-mm thick with 0.08 M TRIS formate buffer (0.07 M TRIS sulfate for the STRs) pH 9.0 and 0.28 M TRIS borate buffer in the 2 % agarose plugs (horizontal, discontinuous electrophoresis system modified after Allen et al. [1]). The electrophoresis was carried out at a temperature of 15 °C (isothermically controlled on a Multiphor II apparatus, Pharmacia LKB) at a constant current (15 mA-25 mA, depending on the width of the gel) with a continuously increasing voltage from 150 to 500 V during the run. Electrophoresis was stopped when the bromphenol blue front had reached the anodal end of the gel.

The DNA fragments were detected by silver staining according to Allen et al. [1]. FGA, ACTBP2, D3S1358, and D21S11 were analyzed by an automated laser fluorescent detection system (A.L.F. DNA sequencer, Pharmacia Biotech): 5 µl of loading dye (formamide, 20 mM EDTA, dextrane blue, self-made internal standard 114 bp or 402 bp M13 PCR fragment) and 1–5 µl of PCR product were mixed and denatured for 3 min at 94 °C, placed on ice, and then loaded on the denaturing polyacrylamide gel (5 % Hydrolink, Long Ranger FMC, 0.5-mm thick and 40-cm long, separation distance 19 cm, 7 M urea, 1 × TBE). The conditions for electrophoresis were set at 34 W, 1500 V, 38 mA, and 3 W laser at 40 °C. Alleles were detected and processed by an automated allele detection software package (A.L.F. Manager and AlleleLinks, Pharmacia Biotech).

Self-made allelic ladders, which were standardized by comparison to sequenced allelic ladders of the stain commission of the German Society of Legal Medicine (GEDNAP exercises) and the AmpFlSTR Blue Allelic Ladder (D3S1358), were used in the gels as references for allele determination. Allele nomenclature was according to the repeat number following the DNA recommendations of the International Society of Forensic Haemogenetics [2, 6, 7, 18].

Results

All DNA extracts were analyzed for nine polymorphic DNA loci on the chromosomes 1, 2, 3, 4, 6, 11, 12, 15, and 21. The exact locations, the names of the polymor-

Table 1 Overview of DNA typing results. VNTR and STR typing results (alleles) of the metastasis, blood, and tumor-free tissue of the first and second recipients. Allele designations were made according to the recommendations of the DNA commission of the International Society of Forensic Haemogenetics, based on the number of repeat sequences

Chromosome localization	Alleles (blood) of the first recipient	Alleles of the metastasis of the first recipient	Alleles of the tumor-free tissue of the second recipient	Alleles of the metastasis of the second recipient	Alleles of the donor
D2p24-p23 (APO B)	37/49	37/37	33/37	33/37	37/37
11 p15.5-p15 (HumTHO1)	9.3/9.3	7/8	7/9.3	7/8/9.3	7/8
D1S80 (pMCT 118)	24/26	18/31	24/31	18/24/31	18/31
15q25-15qter (HumFES)	11/13	12/12	11/12	11/12	11/12
D3S1358	17/18	17/17	14/15	14/15/17	17/17
4q28 (HumFGA)	20/21	20/21	19/22	20/21	20/21
D21S11	65/70	68/70	61/68	61/68/70	68/70
Chr. 6 (HumACTBP2)	20/29.2	17/21.2	16/17	16/17/21.2	17/21.2
12q12-12pter (HumVWA)	15/17	17/18	16/20	16/17/18/20	17/18

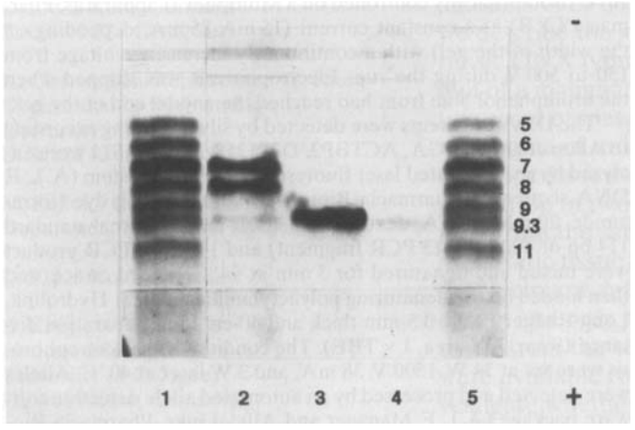

Fig. 1 Electrophoresis of PCR products (HumTHO1) from the first recipient. *Lane 1, 5*: allelic ladder with alleles 5, 6, 7, 8, 9, 9.3, 11; *lane 2*: mixture of metastatic and metastasis-free tissue (alleles 7/8/9.3); *lanes 3, 4*: control blood of the first recipient (alleles 9.3/9.3). In lanes 3 and 4, different amounts of DNA were used

phisms, and the typing results are listed in Table 1. For eight of the nine loci, the blood and the tumor showed different DNA allele combinations and could not have had the same genetic origin. Compared to the organ donor, eight of the nine loci showed a match between the tumor DNA and the donor's type. An electropherogram of PCR products (HumTHO1) is shown in Fig. 1. For the HumFES polymorphism, the tumor reveals a homozygote 12, 12 type, while the donor is a heterozygote 11, 12. In order to evaluate the possibility of a mutation event, as opposed to concluding that the two DNAs were from different individuals, the population frequency of the allele combinations at the eight matching loci was calculated and found to be 1 in 1,000,000,000 German Caucasians. For the second recipient, the DNA results for the tumor tissue showed two alleles at the loci Apo B, HumFES, and HumFGA but three alleles at the loci HumTHO1, D1S80, D3S1358, D21S11, and HumACTBP2, and four alleles at HumVWA. This indicates the presence of a mixture of DNA. The allele pattern can be explained as a combination of the alleles determined for the kidney donor and the second recipient.

Discussion

DNA typing has recently been reported by Beckingham et al. [3] and Wilson et al. [21] as an approach to clarify the origin of tumors after organ transplantation. We analyzed VNTR and STR polymorphisms, which are commonly used in forensic identification of biological material [8, 18], because of the information they provide, their robustness with regard to sample degradation, and their high detection sensitivity. Like many other types of repeated DNA sequences, VNTRs and STRs are located in noncoding regions spread over the whole genome. Compared to the analysis of polymorphic re-

striction sites on chromosome 7 [21], VNTRs and STRs have the advantage of being more polymorphic and independently inherited. A multilocus restriction fragment length detection approach [3] requires high molecular weight DNA and would not have been feasible in our case 2, where the only available tissue was formalin-fixed and paraffin-embedded. Even though it was not possible to extract DNA from an area of tissue that contained only tumor cells, the degree of polymorphism of the tested loci was sufficient to show the presence of alleles from both cell types.

As for cell cultures, the identification of tumor cells has to consider the problem of somatic mutations. Several authors, including Wooster et al. [22] for gastric tumors and Hoff-Olsen et al. for colorectal carcinomas [10], have described the occurrence of such somatic mutations of STRs. The mutations reported never changed the complete genotype but resulted in an additional allele at the specific locus. In our case, twelve independent mutation events would have been required to explain the discrepancy between the first recipient and her tumor DNA, and this is extremely improbable. These two DNA types must have had a separate genetic origin. On the other hand, the loss of HumFES allele 11, which represents the only inconsistency between the donor and the metastasis, could be explained by a mutation event. Since the allele combination over all eight matching loci is as rare as one in a billlion individuals, a mutation in this case is a more likely explanation than the occurrence of an isolated exclusion. This is further confirmed when one looks at the results of the second patient who received a kidney from the same donor. Here, the DNA extract from the tumor showed several DNA alleles that could not have come from the recipient but which were identical to the alleles determined for the first metastasis and the donor. It can, therefore, be concluded that the malignant melanoma was transmitted from the donor to both recipients.

PCR analysis of VNTR and STR polymorphisms yields fast results with minimal amounts of sample material. The accuracy of the test results is not impeded by the occurrence of allele loss caused by mutations or DNA degradation because this can be compensated by extending the number of loci tested in order to either confirm an exclusion or increase the match probability. In conclusion, we have demonstrated the usefulness of forensic DNA typing for determining the genetic origin of metastatic tumors.

Acknowledgement We wish to thank Dr. Mechthild Prinz (Office of the Chief Medical Examiner, NY) for her critical reading and revision of the manuscript.

References

1. Allen RC, Graves G, Budowle B (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. Biotechniques 7: 736–744
2. Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations – further report of the DNA commission of the ISFH regarding the use of short tandem repeat systems. Int J Legal Med 110: 175–176
3. Beckingham IJ, O'Rourke JS, Bishop MC, Ansell ID, Morgan AG, Blamey RW (1994) The use of DNA typing to clarify the origin of metastatic carcinoma after renal transplantation. A clinical and medico-legal problem. Transpl Int 7: 379–381
4. Boerwinkle E, Xiong W, Fourest E, Chan L (1989) Rapid typing of tandemly repeated hypervariable loci by polymerase chain reaction: application to the apolipoprotein B 3' hypervariable region. Proc Natl Acad Sci USA 86: 212–216
5. Brinkmann B (1992) The use of STR's in Stain Analysis Proceedings from the Third International Symposium on Human Identification. Promega Corporation, Madison, pp 357–373
6. DNA recommendations – 1992 report concerning recommendations of the DNA commission of the International Society for Forensic Haemogenetics relating to the use of PCR-based polymorphisms (1992) Int J Legal Med 105: 63–64
7. DNA recommendations – 1994 report concerning further recommendations of the DNA commission of the ISFH regarding PCR-based polymorphisms in STR (short tandem repeat systems (1994) Forensic Sci Int 69: 103–104
8. Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49: 746–756
9. Gill P, Jeffreys AJ, Werret DJ (1985) Forensic application of DNA-fingerprints. Nature 318: 577–579
10. Hoff-Olsen P, Meling GI, Olaisen B (1995) Somatic mutations in VNTR-Locus D1S7 in human colorectal carcinomas are associated with microsatellite instability. Hum Mutat 5: 329–332
11. Kasai K, Nakamura Y, White R (1990) Amplification of a variable number of tandem repeats (VNTR) locus (pMCT 118) by the polymerase chain reaction (PCR) and its application to forensic science. J Forensic Sci 35: 1196–2000
12. Kimpton C, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. Hum Mol Genet 1: 287
13. Li H, Schmidt L, Wei M, Hustad T, Lerman MI, Zbar B, Tory K (1993) Three tetranucleotide polymorphism for loci: D3S1352; D3S1358; D3S1359. Hum Mol Genet 2: 1327
14. Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1: 779
15. Penn I (1991) Donor transmitted disease: cancer.Transplant Proc 23: 2629–2631
16. Polymeropoulus MH, Rath DS, Xiao H, Merril CR (1991) Tetranucleotide repeat polymorphism at the human c-fes/-fps proto-oncogene (FES). Nucleic Acids Res 19: 4018

17. Polymeropoulus MH, Rath DS, Xiao H, Merril CR (1992) Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi-2 (ACTBP2). Nucleic Acids Res 20: 1432
18. Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (1992) Population genetics and forensic efficiency data 4 AMPFLP's. Int J Legal Med 104: 329–333
19. Schmitt C, Schmutzler A, Prinz M, Staak M (1994) High sensitive DNA typing approaches for the analysis of forensic evidence: comparison of nested variable number of tandem repeats (VNTR) amplification and a short tandem repeats (STR) polymorphism. Forensic Sci Int 66: 129–141
20. Sharma V, Litt M (1992) Tetranucleotide repeat polymorphism at the D21S11 locus. Hum Mol Genet 1: 67
21. Wilson LJ, Horvat RT, Tilzer L, Meis AM, Montag L, Huntrakoon M (1992) Identification of donor melanoma in a renal transplant recipient. Diagn Mol Pathol 1: 266–271
22. Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, et al (1994) Instability of short tandem repeats (microsatellites) in human cancers. Nature Genetics 6: 152–156