Utility of Fingernail DNA for Evaluation of Chimerism After Bone Marrow Transplantation and for Diagnostic Testing for Transfusion-Associated Graft-Versus-Host Disease

We report here that fingernails are a useful source of DNA for evaluation of chimerism after allogeneic bone marrow transplantation (BMT) and for diagnostic testing for transfusion-associated graft-versus-host disease (TA-GVHD). Allogeneic BMT has been conducted with increasing success for the treatment of leukemias, severe aplastic anemias, lymphomas, immunodeficiencies, and genetic disorders of hemopoiesis. It is very important to identify the origin of posttransplantation hematopoietic cells to detect disease relapse and graft rejection. However, frequently no recipient original genotype samples are stored after BMT. TA-GVHD is a potentially fatal adverse reaction to blood transfusion with a mortality rate of 90% or higher. We recently developed a definitive diagnostic test for TA-GVHD using highly polymorphic microsatellite markers. The results show the replacement of patient original peripheral blood (PB) DNA types with donor types on development of TA-GVHD. However, samples of patient blood obtained before transfusion are rarely stored until more than 2 weeks after the transfusion when TA-GVHD is usually suspected.

On these occasions, we analyzed polymerase chain reaction (PCR)-amplified fragments of DNA extracted from fingernail clippings to determine the patient original DNA types. DNA extraction from fingernail samples was performed as follows. A fingernail sample of about 10 mg was washed once in washing solution (0.5% sodium dodecyl sulfate (SDS), 0.5 mol/L NaOH), and then twice with distilled water in a 1.5 mL Eppendorf tube. Then it was treated with extract buffer containing 1X TBE (Tris-borate EDTA), 1.4% SDS, 0.15 mol/L NaCl, 28 mmol/L dithiothreitol (DTT) and 68 µg/mL proteinase K. The tube was shaken (about 100 rpm) at 56°C for 3 hours and then the sample was extracted once with phenol/ chloroform and once with chloroform/isoamyl alcohol. DNA was precipitated with isopropyl alcohol and sodium acetate. After centrifugation, the pellet was washed in 70% ethanol, dried, and dissolved in distilled water. DNAs from a fingernail and a test sample were amplified by PCR for several microsatellite loci and applied on polyacrylamide gel to compare the electrophoretic migration patterns. It is known that smaller DNA fragments are obtained from fingernail than from PB samples; however, the fingernail DNA obtained is usually of sufficient quality for enzymatic amplification and genotyping. In our experiments, the electrophoretic patterns of the fingernail DNA samples were identical to those of the PB DNA samples obtained from 15 normal controls and 49 non-TA-GVHD patients, indicating that fingernail DNA can also be used to determine the individual-specific genotype.

In BMT, a successful outcome has been associated with a state of stable chimerism in which all the bone marrow cells are of donor origin. A representative example of complete chimerism at the vWF locus is shown in Fig 1A. Figure 1B shows an example of graft rejection detected by analysis of the APO locus. In an other case,
relapse after BMT was detected by analysis of the CD4 locus\(^5\) (Fig 1C).

PB DNA types determined after patients developed symptoms of TA-GVHD have been found to differ from the fingernail DNA types. In other words, the patients' peripheral white blood cells had been replaced by exogenous lymphocytes during the development of TA-GVHD. In one case in the present study, the patient peripheral blood DNA types differed from the fingernail DNA types at the HGH\(^6\) and APO loci (Fig 1D). Furthermore, the DNA types of the patient PB samples were identical to those of the PB sample from one of the four blood donors. Fingernail samples can be obtained without any injury to the patient and are easy to preserve and transport.

These results show that use of fingernail DNA is useful for evaluation of chimerism after BMT and for diagnostic testing for TA-GVHD.

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REFERENCES


Fig 1. DNA polymorphism analysis in post-BMT patients and a TA-GVHD patient. Microsatellite bands obtained by PCR of DNA samples separated on 7% polyacrylamide gels. The amplified DNA fragments were observed with silver staining. (A, B, and C) The PCR products obtained using the primer sets for vWF, APO and CD4, respectively. Lane 1, DNA size markers; Lane 2, PCR products derived from DNA extracted from the post-BMT patient fingernail sample; Lane 3, PCR products derived from DNA extracted from the BMT donor fingernail sample; Lane 4, PCR products derived from DNA extracted from a sample of the post-BMT patient's PB mononuclear cells; Lane 5, PCR products derived from DNA extracted from a sample of the post-BMT patient's PB granulocytes. (D) the PCR products obtained using the primer sets for HGH (Lane 2 through 4) and APO (Lane 5 through 7). Lane 1, DNA size markers; Lanes 2 and 5, the PCR products derived from DNA extracted from the TA-GVHD patient fingernail sample; Lanes 3 and 6, the PCR products derived from DNA extracted from a sample of the patient's PB obtained after the patient developed symptoms of TA-GVHD; Lane 4 and 7, the PCR products obtained using DNA extracted from a sample of the blood donor's PB.
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