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Platelet transfusion can mimic somatic mtDNA mutations

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Chemotherapy-induced mitochondrial DNA (mtDNA) alterations are well known in patients treated for viral infections with nucleoside analogue reverse transcriptase inhibitors.[1] It is also hypothesized that certain chemotherapeutic agents for treatment of cancer may cause mtDNA mutations *in vitro*.[2,3] A study by Wardell *et al*. reported mtDNA mutations after chemotherapy and irradiation several years after the end of therapy. Although an increase in mtDNA mutations was observed in this study, the level of heteroplasmy (a mixture of mutated and normal mtDNA molecules in one cell) was usually below 2%.[4] The mitochondrion contains multiple copies of DNA molecules per cell; therefore, it is unlikely that random mutations induced by cytotoxic agents would affect a high number of genome copies. Accordingly, therapy-induced mutations were present at a low level.[4]

Recently, Carew *et al*.[5] reported a high mtDNA mutation rate in leukocytes of patients with chronic lymphatic leukemia (CLL) after chemotherapy. In this study, the mtDNA sequences of primary CLL cells were compared to the Cambridge reference sequence. As expected, all patients displayed a variety of homoplasmic differences to the reference sequence of the mtDNA. No difference in the frequency of homoplasmic mutations was observed in the untreated and treated group. However, CLL cells with prior chemotherapy had a significant higher frequency of heteroplasmic mutations than those of untreated individuals.[5] In all cases, a high level of heteroplasmy between 30 and 70% was detected.

A similar observation was made in our laboratory in a patient with a relapse of acute lymphoblastic leukemia (ALL) (male, age 6 years). The mutation screening of the complete mtDNA revealed 19 acquired somatic heteroplasmies in peripheral blood mononucleoar cells (PBMC) 4 weeks after the onset of therapy (data not shown). The therapy regimen (Berlin–Frankfurt–Münster (ALL-BFM) protocol) was identical to the one in the other 11 patients treated for ALL in our hospital, where we did not detect an increased number of somatic heteroplasmic mtDNA mutations in PBMC after chemotherapy (data not shown). Further evaluation of disease history and treatment revealed that the ALL patient described above had received a platelet concentrate 3 days prior to sampling of the patients blood. Unfortunately, at that time point, the patient was not available for additional investigations.

To test our hypothesis, that donor platelet contamination might have mimicked the induction of heteroplasmic mtDNA mutations in our patient, we investigated mtDNA mutations of PBMCs of patients prior and after single donor platelet infusion. White blood cells were isolated by selective lysis of red blood

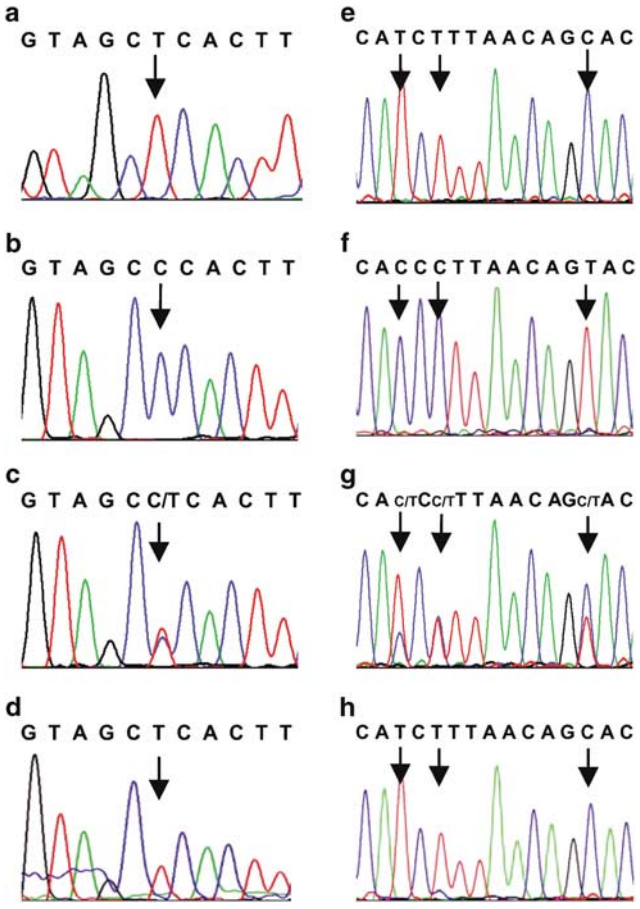

Figure 1 MtDNA analysis of a patient prior and post-infusion of platelets of a single donor. The first blood sample was collected at the same day before the transfusion of the platelet-concentrate, the second 1 h after the transfusion and a third blood sample was collected 2 weeks later. Samples from patients were collected after informed consent was given. Two regions of the mtDNA, containing parts of the COI (6747–7088) gene and D-loop (16098–16456), were amplified and sequenced as previously reported.[7]Sequence analysis of the first PCR fragment (**a**–**d**) showed a 7028T variation in the blood sample before the transfusion (**a**), and a 7028C variation in the donor platelets (**b**). The blood sample drawn 1 h after the injection represents both variants at 7028 (**c**). Sequence analysis of the second PCR fragment of the patients blood sample before the injection showed 16213G, 16273C, 16294T, 16296T and 16304C, and the donor platelets 16213A, 16273T, 16294C, 16296C and 16304T. Only the last three mutations are shown in the sequence (**e**–**h**). The blood sample drawn 1 h after the injection presented with the following mixtures 16213G/A, 16273C/T, 16294T/C, 16296T/C and 16304C/T (**g**). After 20 days, the sequence variants of the donor mtDNA disappeared (**d**, **h**).

cells and digested with proteinase K before PCR amplification. DHPLC analysis and sequencing of the mtDNA revealed heteroplasmy of patient blood DNA preparation due to the presence of donor DNA of contaminating platelets (Figure 1). In agreement with a recent study, we observed that even the enrichment of PBMC by Ficoll Paque® (Pharmacia Biotech, Uppsala, Sweden) gradient did not substantially eliminate the donor platelet contamination.[6]

Full clearance of donor mtDNA was observed after 3 weeks (Figure 1).

The degree of heteroplasmy we noticed after platelet infusion (30–70%) (Figure 1) was similar to that detected in most patients in the study of Carew *et al*.[5] Since platelet counts are frequently low in CLL patients repeated platelet transfusions are necessary. The untreated group of patients in the previous study had an average lower disease stage and therefore might not have received platelets within 3 weeks prior to sampling. In contrast, toxicity of chemotherapy leads frequently to thrombocytopenia and subsequent treatment with platelet concentrates.

There is no indication in the previous paper that patients with prior platelet infusions were excluded from the study or if the blood samples were taken at a certain interval after the end of therapy or platelet infusion. However, the very high frequency of high level heteroplasmic mutations in some of the treated patients, as well as the almost identical picture we obtained after sequence analysis of patients following platelet infusion, led us to the conclusion that this is rather caused by donor platelets and unlikely the consequence of chemotherapy.

Determination of mtDNA mutations can only be reliably performed at least 3 weeks after administration of blood products to avoid detection of donor mtDNA. Alternatively, a recently described procedure using special conditions for blood collection to eliminate disturbing platelets could be employed for isolation of PBMC.[6]

The high level of heteroplasmy observed by Carew *et al*. could be explained by selective advantage. However, we would not expect that in one patient, 12 distinct mutations can have a selective advantage leading to similar high levels of heteroplasmy. Especially for synonymous heteroplasmic mutations a selective pressure is most unlikely.

Epidemiological and geographic differences between individuals complicate the statistical interpretation of mtDNA mutations if they are compared to the Cambridge reference sequence. Therefore, analysis of therapy-induced mtDNA mutations should be carried out on matched patients samples. Ideally, buccal swabs, urine sediment or normal leucocytes isolated by FACS sorting before initiation of therapy would be the most appropriate reference material for screening of chemotherapy-induced mtDNA mutations.

In conclusion, analysis of mtDNA mutations in peripheral blood samples from patients with a recent history of donor platelet transfusion should be interpreted consciously.

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Clonotypic analysis of acute lymphoblastic leukemia with a double *TEL-AML1* fusion at onset and relapse

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The *TEL-AML1* (*ETV6-RUNX1*) fusion is the most common genetic alteration in childhood acute lymphoblastic leukemia (ALL).[1,2] Lines of evidence demonstrated the prenatal origins of *TEL-AML1* and the requirement of additional complementary genetic events leading to overt leukemia.[3,4] Ford *et al*[5] and Konrad *et al*[6] recently demonstrated that *TEL-AML1*-positive ALL cells in late relapse were derived from a preleukemic clone not eliminated by initial chemotherapy, suggesting that additional molecular events within the common preleukemic clone give rise to a new leukemia as a conventional relapse. A recent report from Austrian BFM cooperative study group revealed that duplication of *TEL-AML1* was observed in approximately 15% of *TEL-AML1*-positive ALL cases as one of the secondary genetic abnormalities.[7] However, it remains uncertain whether or not duplication of *TEL-AML1* is sufficient to generate overt leukemia. To clarify this point, the clonotypic analysis of late relapsed cases with double *TEL-AML1* fusion would be informative.