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The existence and role of microchimerism after microtransplantion

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ABSTRACT

Aim: To study microchimerism's role and function after microtransplantation and identify novel genetic markers for microchimerism detection.
Methods: Analyzing microchimerisms from patients microtransplanted to determine the presence of *GSTT1*, *GSTM1*, *SRY* and other genetic markers by real-time PCR.
Results: Microchimerism could be detected for a short time after microtransplantation simultaneously with hematopoietic recovery. In conclusion, microchimerism might accelerate hematopoietic recovery and *GSTT1* and *GSTM1* genes could be used as genetic markers to differentiate donor cells.
Discussion: Microchimerism could exist for a short time after microtransplantation and appears to function in hematopoietic recovery. According to published reports, cytokines secreted from microchimerisms could be detected in recipients and exhibit some function on the host. Therefore, cytokines secreted from donor cells are hypothesized to accelerate hematopoietic recovery. The evidence to prove a longer existence for microchimerism is insufficient and needs supports by additional experiments; however, we cannot deny its existence just because of the limited sensitivity of methods.

1. Introduction

Microtransplantation is a new therapy protocol for elderly leukemia patients proposed by Mei Guo et al. in 2011, in which patients receive anti-leukemia immunity similar to that with transplantation while effectively avoiding graft-versus-host disease (GVHD). In this published study, 58 Acute Myelocytic Leukemia (AML) patients over the age of 60 with no HLA-identical sibling donor were randomly assigned to either the test group or the control group. The patients in the control group were treated with standard chemotherapy, and those in the test group were infused with HLA-mismatched, granulocyte-macrophage colony stimulating factor mobilized peripheral blood stem cells from a relative after receiving the same chemotherapy regimen. The results showed that both the complete remission rate and the 2-year overall survival rate were higher in the test group than in the control group: 80% vs. 43% and 39% vs. 10%, respectively. The median recovery times for neutrophils and platelets decreased dramatically in the test group compared with the control group (11 days vs. 16 days and 14.5 days vs. 20 days, respectively), and no patients contracted GVHD when graft-versus-leukemia effect (GVL) was still present [1]. This published study evoked great interest among numerous hematologists. The mechanism by which microtransplantation creates such exciting results is not fully understood. Hematopoietic stem cell transplantation cures leukemia via the GVL effect with donor chimerism. Therefore, the possibility exists that the curative mechanism of microtransplantation requires microchimerism.

Microchimerism is the presence of a minimal number of cells or DNA in another genetically distinct body and can be caused by natural or iatrogenic processes (such as transplantations and blood transfusions). The most common manifestation of microchimerism occurs during the process of genetic material exchange between the mother and fetus during pregnancy. Researchers have found that natural microchimerism could exist for long periods of time and may play an important role in treating autoimmune diseases and tumors [2–4]. According to certain studies, microchimerism achieves this function via cytokine secretion by donor cells. For example, in 2007, Wrenshall et al. proved using a genetic knockout mouse that fetal microchimeric cells in the mother rat produced IL-2 [5]. These authors proposed that microchimerism is the key component of the microtransplantation

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mechanism of action. In an early study of microtransplantation, the gene sex determining region Y (*SRY*) was the only genetic marker that could be used to detect microchimerism; thus, these studies were confined to female recipients with male donors and were therefore greatly restricted. In 2005, Velasco et al. utilized the double allele insertion/deletion (In/Del) polymorphism gene glutathione-S-transferase mu 1 (*GSTM1*), theta 1 (*GSTT1*) et al. as the genetic marker for differentiating the donor from the recipient and detected donor chimerism using real-time PCR after transplantation [6–9]. They obtained a peak intensity of 10^{-5}. From the genetic markers they used, we selected specific novel genetic markers, such as *GSTT1* and *GSTM1*, to assess donor microchimerism using patient DNA specimens preserved after microtransplantation. We expected to determine the incidence of microchimerism prior to and after microtransplantation and to perform a preliminary study of its function.

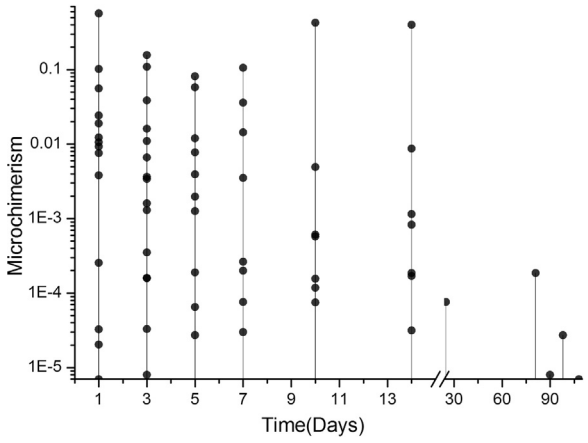

Fig. 1. Post-microtransplantation microchimerism assessment of a genetic marker by real-time PCR. It seems that most of the microchimerism exists in the first 14 days after the cell infusion, and the shorter of the period for the detection after the cell infusion, the more possible to detect the microchimerism effectively.

2. Patients, materials, and methods

2.1. Patients and samples

All 12 patients underwent microtransplantation in Affiliated Hospital of Academy of Military Medical Sciences; these included 10 AML patients and 2 ALL patients. All the donor/recipient pairs were HLA-mismatched. We assayed for microchimerism continuously in the 12 patients at different time points post-transplant, i.e., on days1, 3, 5, 6, 10, and 14as well as after 30days. We extracted high-molecular weight DNA from the peripheral blood or bone marrow nuclear cells using a DNA extraction kit, evaluated the concentration and purity of each DNA sample with a UV spectrophotometer, and stored the samples at −20 °C.

2.2. Genetic marker selection and primer and probe design

We retrieved the sequences provided by Velasco and downloaded the insertion/deletion sequence of *GSTT1* and of other genes from the human In/Del polymorphism database (http://www.ncbi.nlm.nih.gov/snp/). With the In/Del sequences, we designed specific primers and probes for each selected insertion/deletion genetic polymorphism marker using ABI Primer Express 3.0 (Table 1). The specificity of the amplified genes was achieved using a specific primer designed based on the polymorphic site of the genetic marker; the second primer and probe were designed against the common area of the base sequence. The parameters for the qualitative PCR cycles were consistent with those for quantitative amplification. The primers and probes for the 12 genetic markers were diluted in ddH_2O at the same concentrations and were stored at −40 °C.

2.3. Real-time PCR

A STRATAGENE MX3005P was utilized for real-time PCR. The PCR conditions were as follows: (1) Reaction mixture: 25 μl Master Mix 2X Buffer (Applied Biosystems, USA), 200 nM forward and reverse primers, 100 nM probe, and 100 ng DNA) PCR cycling: denaturation at 95 °C for 5 min, and 40 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 30 s. All the genetic markers were amplified in the donor and recipient DNA specimens prior to microtransplantation to determine which one was applicable. A 100-ng DNA sample was added to each amplification tube and was amplified using the same amplification program. There was at least one negative control sample for each amplification reaction. After amplification, the Ct values for the positive specimens were between 20 and 26, and all the values for the negative specimens were 0. Negative values for the recipient and positive values for the donor provided important information regarding certain genetic markers. Because of the quantitative analyses were performed regardless of the utilized genetic marker (as illustrated by the fluorescent quantitative PCR in the Results section), if more than one genetic marker was informative, only one was used. Pre-microtransplantation (recipient and donor) and post-MST (recipient only) DNA samples were analyzed for microchimerism. Recipient-specific alleles, donor-specific alleles, and standard genetic plasmids were amplified in a single experiment. We chose β-actin as the reference gene and amplified it using a specific primer/probe set simultaneous to amplifying the target gene in the same specimen. The Ct value of each sample was obtained by real-time PCR after sample amplification. The effective amounts of DNA were individually normalized in each sample by comparison with the Ct values of the reference gene in the real-time fluorescent quantitative PCR results as well as with those obtained by PCR amplification of genetic markers in each recipient and donor sample.

3. Results

3.1. Utility and sensitivity of genetic markers

We designed primers and probes for *GSTT1* and *GSTM1*, as well as for other genes, and obtained 12 specific genetic markers that were located at 12 loci on 10 different chromosomes. The primers and probes for the 12 genetic markers are listed in Table 1. To evaluate the ability of these 12 genetic markers to distinguish between the donor and the recipient, we analyzed 50 pre-transplantation DNA specimens from the 25 donor/recipient pairs. The number of informative sites for each donor/recipient pair ranged from 1 to 7(average, 3.6); 21 pairs were suitable for microchimerism detection with qualified informative sites. Genetic markers on the selected alleles could distinguish 84% of the matched donor/recipient pairs.

In the in vitro simulation experiment, a donor DNA sample was mixed with a recipient DNA sample that had been diluted 1:10. The amplification curve shifted to the right when the number of added DNA copies decreased. A 10-fold decrease in the number of DNA copies elicited a 3-Ct increase, as expected. Based on the amplification curve in Fig. 1, a sensitivity of 10^{-5} was achieved for the DNA samples, which was consistent with the literature.