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Effectiveness and limitations of resolving HLA class I and class II by heterozygous ambiguity resolving primers (HARPs)—a modified technique of sequence-based typing (SBT)

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ABSTRACT

Objectives: The aim was to evaluate the use of combination of SBT (sequence based typing) and HARP (heterozygous ambiguity resolving primer) in HLA typing to acquire high resolution typing results.

Design and methods: 167 DNA samples were analyzed by SBT. The web site HARPs Finder provided by Conexio Genomics, the developer of HARPs (http://www.harpsfinder.conexio-genomics.com/index.html) was then used to search for appropriate HARPs.

Results: HARPs can resolve 95% of ambiguities for locus A; 86% for B and 60% for DRB1 locus. However, there are still limitations. Practically PCR products of un-separated alleles are used as templates for sequencing by HARP; sometimes, it is still impossible to get unambiguous typing.

Conclusions: We outlined the advantages and disadvantages of SBT/HARP. A list of HARPs for choice to resolve ambiguity of SBT in Taiwanese population is concluded.

Introduction

Human leukocyte antigen (HLA) plays an important role in modern medical and clinical science, involving in many fields such as immune system function, disease defense and reproduction. As of today, the numbers of HLA class I and class II alleles have reached 6403 as dated in April 2011 on IMGT. For class I, there are 4946 alleles, and 1457 alleles for class II [1]. In allogeneic organ or hematopoietic stem cell transplantation, the differences in HLA typing for donor and recipient could induce acute or chronic rejection, which might lead to transplantation failure or, in more serious cases, fatal consequence [2]. It is now widely accepted that high-resolution HLA typing is required for identification and selection of best-matched donor in order to minimize the probabilities of rejection.

There are two main categories of HLA typing methodology: serological-based and molecular-based technique. Lots of studies indicate that serological technique may be unsatisfactory or unreliable in resolving HLA alleles in high resolution compared to molecular methods [3–5]. The most commonly used molecular HLA typing methods include sequence specific oligonucleotide probe (PCR-SSO), sequence specific primer (SSP) and sequence-based typing (SBT). PCR-SSO can be a high-throughput and relatively inexpensive method, so it is usually used for large-scale, low-resolution HLA allele analysis involving large numbers of samples. SSP is typically used on samples that have failed to be analyzed by SSO, serological method or for situation that higher resolution is required [6]. Among all molecular typing techniques, SBT has the highest resolution and is the only way to directly sequence and identify new alleles. However, conventional SBT technique is much complicated and more expensive than PCR-SSO and SSP; thus its use as routine HLA typing in clinical lab is limited. However, recent developments and advances on automated genetic sequencer, labeled-dye chemistry and alignment analysis algorithms make it possible to regard SBT as part of the routine HLA-typing repertoire.

Because of the advance in HLA typing methodologies, total number of HLA alleles increases rapidly over the past couple of years; hence the complexity of HLA allele ambiguities grows rapidly as well [7]. It is becoming more and more difficult to employ a single HLA typing technique to resolve different allele combinations. Commonly the SBT reagents amplify and sequence at least exon 2 and exon 3 of HLA class I as well as exon 2 of HLA class II. Even though SBT is the technique with highest resolution, it could encounter ambiguities in resolving heterozygous allele pairs. Ambiguous alleles occur when sequenced by common sequencing primers and the analyzing software cannot decide whether it is a cis or trans combination of

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polymorphic sites. For other times, ambiguities occur when there is only one DNA base different in two typing. Typically, a panel of specially designed SSP or an additional group-specific PCR amplification (GSA) followed by sequencing is employed to identify those ambiguities. The use of these supplemental methods could decrease HLA typing throughput and efficiency, could increase the probability of operation error and is also time-consuming. A modified SBT technique, heterozygous ambiguity resolving primer (HARP), is used to resolve cis/trans allele combinations in a one-step manner to avoid the drawbacks in group-specific PCR amplification or SSP.

HARPs work differently from group-specific PCR amplification primers or cloning in resolving polymorphism in allele combinations. GSAs are primers which can only amplify certain alleles in the PCR phase of SBT; thus, only one allele shall be sequenced in the following sequencing phase. HARPs work in the sequencing phase and were specially designed in their annealing sequence to avoid sequencing of both alleles in the mixture of PCR products. Hence, allele separation is unnecessary, and extra processing time can be saved.

Fig. 1 illustrates the principle of HARP. HLA class I is utilized here as an example. Yellow and green bars represent exon 2 and 3; purple vertical lines within the bar are the polymorphic sites. Two different HARPs (as labeled HARP 1 and 2) recognize specific polymorphic site, allowing for the subsequent haplotype sequencing to discriminate heterozygous allele combinations.

In some ambiguous allele combinations, only one HARP might be required. For example, HARP 2 only anneals to one of the two alleles and reads out the sequence on exon 3 for that particular allele. The sequenced DNA alignment is compared to already existing DNA alignment on IMGT for identification. As a result, correct typing is obtained.

Using HARP is a new way for resolving heterozygous ambiguity in sequence-based typing. It works in the same way as the sequencing primers, except one difference, that HARP only sequence one of the two alleles. Hence no allele separation before sequencing reaction is required.

Human leukocyte antigen (HLA) typing is a requirement for matching of patient and donor in cord blood and bone marrow transplantation. The match between patient and donor will influence the successfulness of transplantation. SBT/HARP is one of the ultimate solutions for high resolution HLA typing.

Materials and methods

Blood

The sources of samples include cord blood units from our lab and specimens of international proficiency test/cell exchange program.

DNA isolation

Genomic DNA was extracted by NucleoSpin Blood kit (Macherey–Nagel GmbH & Co. KG, Düren, Germany).

Low and intermediate-resolution HLA typing for cord blood units and DNA samples

The low resolution HLA typing was performed by PCR-SSO (GenProbe Transplant Diagnostics, Stamford, CT, USA) and Luminex technology (Luminex Corporation, Austin, TX, USA). Samples displaying uncommon heterozygous allele combinations or those failing to generate SSO results were subsequently tested with PCR-SSP (Olerup SSP kits, Olerup SSP AB, Sweden) to obtain intermediate resolution for both HLA class I and class II.

Genomic sequence-based typing for HLA class I and class II genes

For cord blood units failed to be resolved either by SSO or SSP because of single nucleotide polymorphism in variable regions, SBT for HLA-A, B and DRB1 locus was performed to obtain highest resolution, unambiguous results. Complete PCR amplification and sequencing reaction for exon 2 and exon 3 of HLA class I as well as exon 2 of HLA class II was performed by reagents and primers provided by AlleleSEQR (Abbott, Germany, Fig. 2). The optimal PCR and purification conditions

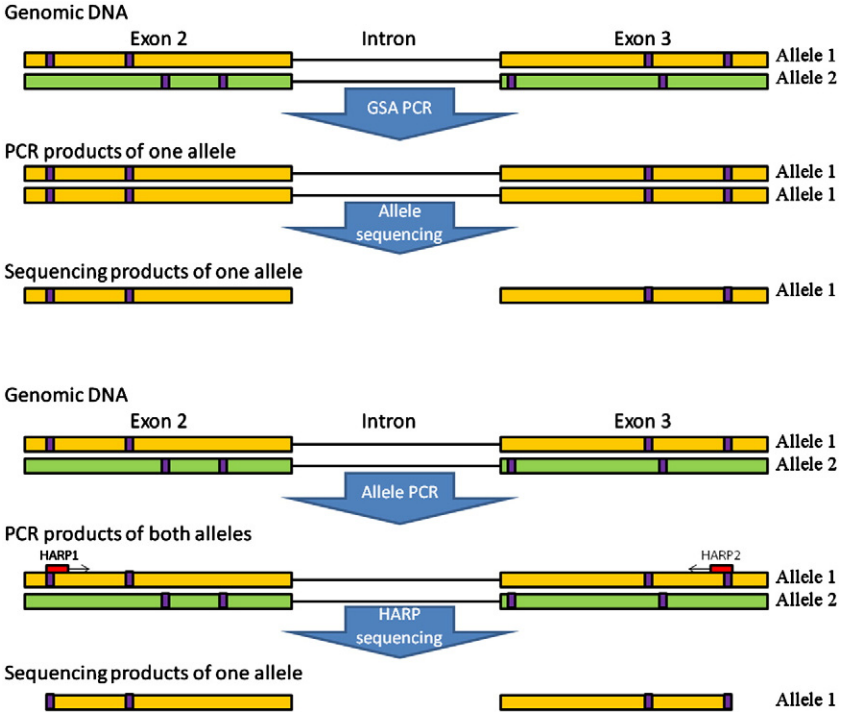

Fig. 1. The difference on actions of GSA and HARP. HLA class I is utilized here as an example. Yellow and green bars represent exons on different chromosome; purple vertical lines within the bar are the polymorphic sites. Two different HARPs (as labeled HARP 1 and 2) recognize specific polymorphic site, allowing for the subsequent haplotype sequencing to discriminate heterozygous allele combinations.