Секция «Тезисы на английском языке»

STR-PCR based monitoring of chimerism requires correct calculation due to the PCR stutter-bands contribution

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After the allogeneic bone marrow transplantation it is necessary to control engraftment by chimerism monitoring. Routine clinical test is based on differences in the length of short tandem repeats (STR) from the donor and the recipient [1]. Calculation may be complicated by formation of stutter products during polymerase chain reaction (PCR). Stutter products are sequences which are 4 nucleotides shorter than a specific marker and may interfere with a specific sequence of recipient's DNA hindering chimerism estimation based on that locus [3].

One may suggest to use markers with stutter-peaks only if stutter-bands are comparable with donor allele peak height. [2]

The aim of the study was to identify the contribution of stutter-bands to the total amount of PCR-product and to derive universal formulae for the chimerism calculation excluding stutter percentage.

Methods: Genomic DNAs were isolated from donors and patients bone marrow samples. Chimerism was assessed by the STR- PCR analysis with COrDIS Plus multiplex kit for amplification of 19 polymorphic STR-markers and amelogenin loci. The fragment analysis was performed on a 3130 Genetic Analyzer. The data processing was accomplished using GeneMapper v.4-0 software. Informative loci were chosen beforehand comparing pretransplant patient DNA and donor DNA. The percentage of donor chimerism as well as stutter percentage was calculated using standard formula [2].

Results: 50 markers (18 homo-, 15 heterozygous and 17 heterozygous with one stutter visible only) (Fig. 1) were evaluated and the contribution of stutter peak to the total amount of product was estimated to vary from 1.2% to 11% and was shown to be locus-specific constant (considering number of informative markers and stutter peaks) with invariable PCR protocol (calculations were performed for the 10 loci DNA from 11 patients). SD in each locus was equal or less than 1.5% for each combination of alleles.

Formulae for recipient DNA percentage were derived (Fig.1). Formulae were tested on the bone marrow samples of patients with mixed chimerism (with using "stutter-free" informative markers as a control). The results of chimerism estimation based on "stutter-complicated" markers (using proposed formulae) and conventional "stutter-free" markers appeared to be the same.

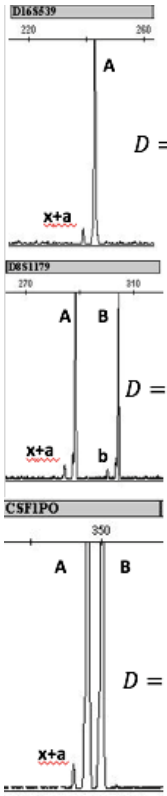
Conclusion: In case of stutter-free donor's alleles absence the calculation of chimerism can use informative markers coinciding with stutter-peaks. For correct calculation the formulae given above should be used.

Источники и литература

1) Blau OV Chimerism after allogeneic hematopoietic stem cell transplantation. Clinical oncohematology 2013, 6 (1): 34-39
2) Nollet F, Billiet J, Selleslag D, Criel A: Standardization of multiplex fluorescent short tandem repeat analysis for chimerism testing. Bone Marrow Transplant 2001, 28:511–8

3) Walsh PS, Fildes NJ, Reynolds R. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. Nucleic Acids Res. 1996 Jul 15;24(14):2807-12

Иллюстрации



$$\text{Actual recipient's \%} = \frac{\text{apparent rec./total DNA ratio - stutter/total DNA ratio}}{\text{1-stutter/total DNA ratio}} * 100\%$$

$$D = \frac{\frac{x+a}{x+a+A} - \frac{a}{a+A}}{1 - \frac{a}{a+A}} * 100\%$$

$$D = \frac{\frac{x+a+b}{x+a+b+A+B} - \frac{a+b}{a+b+A+B}}{1 - \frac{a+b}{a+b+A+B}} * 100\%$$

$$D = \frac{\frac{x+a}{x+a+b+A+B} - \frac{a}{a+b+A+B}}{1 - \frac{a}{a+b+A+B}} * 100\%$$

** D is percentage of recipient chimerism*

A and B are donor allele peaks heights, a and b — donor stutter-bands heights, x -recipient marker peak height (in case of heterozygote it seems as x_1+x_2) (x+a) is a peak height when recipient`s marker coincides with donor stutter band; aa+Aand other constant relations were calculating by donor`s DNA usage.

*** The presented formulas are usable for alleles not shared between donor and recipient to calculate chimerism values. The similar formulas were derived for one shared and one unshared allele for calculation of chimerism values*

Рис. 1. Formulas for recipient DNA percentage