Study of Donor Chimerism

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The outcome of a stem cell transplant can be complete donor chimerism (100% donor cells) or mixed donor chimerism of varying proportions of donor and recipient cells. Another type of chimerism called split chimerism may also exist in which one or more whole lineage is of recipient and the other of donor in origin.

The haematopoietic cells of donor origin can be detected in the host. The test may be done on peripheral blood, bone marrow or lineage specific cells e.g. T cells, B cells and granulocytes. Peripheral blood is equally sensitive in detection of chimerism than bone marrow. The study of donor chimerism may be done to know:

- Whether the donor engraftment has occurred or not?
- Whether there is mixed chimerism? If present then how much?
- If there is mixed chimerism then which lineages are mixed and which are fully donor?
- Whether there is chimerism in the lymphoid and the myeloid compartments?

Applications

- Myeloablative Stem Cell Transplant
  - Donor engraftment
  - Graft rejection
  - Prediction of GVHD
- Non-Myeloablative Stem Cell Transplant (Mini transplant)
- Relapse prediction

Techniques

Donor chimerism is usually tested by cytogenetics/FISH, real time PCR or STR analysis. STR analysis is basically an extension of the analysis of DNA mixtures discussed in Chapter 9 and 15.

Samples required

1. Recipient's pre-transplant blood sample in EDTA. If this is not available recipient's buccal mucosal cells, skin biopsy or hair roots may be collected on a stick swab to represent the pre-transplant status. Care should be taken to avoid contamination of the swab with recipient's blood.
2. Donor blood in EDTA.
3. Recipient's post transplant blood or bone marrow sample.

Procedure

1. Extract DNA from the three samples.
2. The STR profiling may be done by manual method or by genetic analyzer (Chapter 9).
3. Out of the many STR markers "informative" marker(s) are chosen. The STR marker is called "informative" when its alleles can distinguish between the recipient and the donor DNA. For example if at the D21S11 locus the recipient has alleles 28,29 and the donor has 28,31 the marker is informative because allele 29 and 31 are exclusive for the recipient and the donor respectively.
4. In the presence of complete donor chimerism the recipient's post transplant DNA shows the donor's genotype. In mixed chimerism mixture of recipient and donor genotypes is seen (Fig 17.1).
5. In the manual method quantitative estimation of amplified products is done by densitometry of polyacrylamide gels (Fig 17.2).
6. If genetic analyzer is used for STR genotyping the analysis is done by measuring the peak heights and area under the curve.
7.

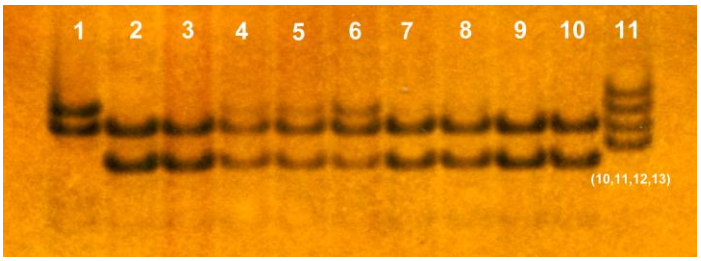

Fig. 17.1. PAGE of PCR amplification at D5S818 locus. Lane 11 shows allelic ladder with alleles 10-13. The lane 1 shows recipient's pre-transplant sample (alleles 11,12) and lane 2 shows donor sample (alleles 9,11). Lane 3-10 show serially collected recipient's post-transplant samples. Lanes 4-6 show gradually appearing recipient's exclusive allele (12) and a gradually decreasing strength of the donor' exclusive allele (9) indicating graft failure. The patient received an infusion of donor lymphocytes that resulted in disappearance of the recipient's allele (lanes 7-10).

Calculation of donor and recipient component

Calculation of the donor and recipient components is usually done by analyzing the samples on a genetic analyzer and then measuring the peak areas. A cost effective alternate is by doing PAGE followed by densitometry. Figs. 17.2 and 17.3 show an example of the calculation of donor chimerism by PAGE and its densitometry at the D5S818 locus.

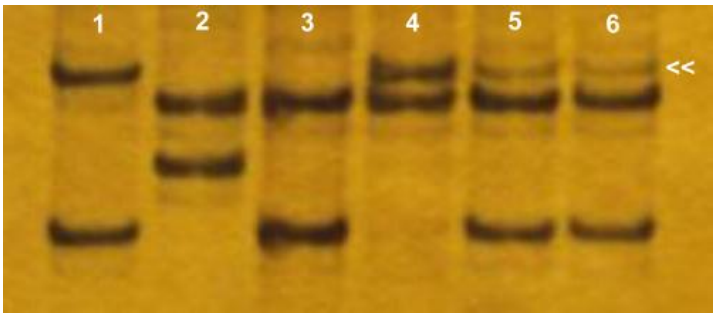

Fig. 17.2. PAGE of STR amplification at D5S818 locus. Lanes 1-4 show the samples of father, mother, donor and recipient (pre-transplant) respectively. Lanes 5 & 6 show the recipient's post-transplant samples in duplicate. The sample shows mixed donor chimerism represented by reappearance of the recipient's exclusive allele (arrow).

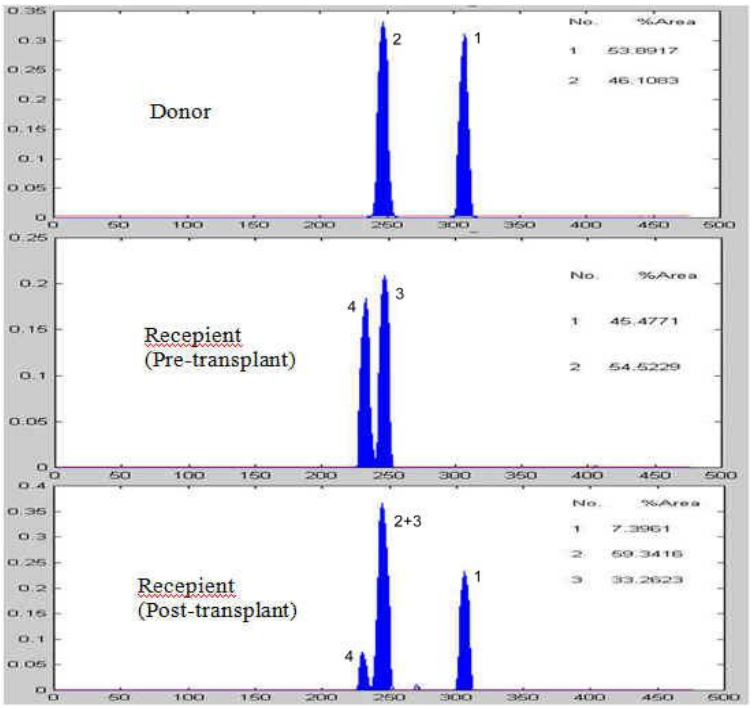

Fig. 17.3. Densitometric recording of STR amplified products run on polyacrylamide gel shown in Fig. 17.2. The recipient's post transplant sample shows mixed donor chimerism.

The recipient's pre transplant allele peak 4 represents 7.3%. The total recipient's component is calculated by doubling the allele peak 4 component (3+4) i.e. 14.6%.

Real time PCR for assessing donor chimerism

As discussed in Chapter 6 end point analysis of PCR products by gel electrophoresis, including analysis on a genetic analyzer, is not very good for quantitative assessment of DNA. Qantitative assessment of donor chimerism by real time PCR can give a more accurate measurement of the donor or recipient components. SNP genotyping by real time PCR using TaqMan® probe assay has been used for this purpose.

Bibliography

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