Int J Clin Lab Res 21:269-272, 1992

International Journal of Clinical & Laboratory RESEARCH

© Springer-Verlag 1992

Reviews

DNA fingerprinting in leukemia

Seppo Pakkala

Transplantation Laboratory, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, Finland

Summary. The detection of polymorphic regions of the human DNA provides individual specific markers, DNA fingerprints (DNA-F), for the identification of individuals. DNA-F can also be used for the detection of cell clones and serve as molecular markers of malignant disease. In leukemia DNA-F analysis can be used for the follow-up studies during remission or after bone marrow transplantation. Recent studies on DNA fingerprinting in leukemia are reviewed.

Detection of minimal residual disease is a major challenge in leukemia research. In acute leukemia 60%-85% of patients achieve remission with intensive chemotherapy, but only 20%-30% will have over 5 years of disease-free survival. To improve long-term results, residual leukemia should be detected early during remission and treatment modified before overt relapse. Several methods have been proposed for this purpose, but few have proved useful since most leukemia cells do not have characteristics which can be used at the single-cell level to demonstrate the leukemic origin of the cell [4, 7, 16, 27, 31].

Molecular biological techniques provide new tools for accurate detection of small numbers of cells [1, 8, 32, 33]. The DNA fragments produced by digestion with restriction enzymes and detected by minisatellite probes are highly specific for each individual – the so called DNA fingerprints (DNA-F). DNA-F analysis is based on restriction fragment length polymorphism caused by small changes in DNA, base substitutions, or deletions, which create or destroy specific endonuclease cleavage sites [2]. This results in differences among alleles in the length of a particular restriction fragment; these differences can be detected after restriction endonuclease digestion by Southern hybridization with a specific probe. The minisatellites are short nucleotide sequences which are found as tandem repeats throughout the genome in multiple different loci. The allelic polymorphism results from differences in the number of repeats as the result of unequal mitotic or meiotic exchange of DNA [12]. These areas represent several different loci in the human genome and it is thus possible to study changes in numerous loci simultaneously by these probes. Jeffreys et al. [13, 14] have shown that the minisatellite fragments are mainly autosomal in origin and are somatically stable, thus DNA isolated from different normal tissues of an individual always displays the same DNA-F pattern. The mendelian inheritance, somatic stability, and low mutation rate make them suitable for the identification of haplotypes or individuals; DNA fingerprinting can be used to demonstrate engraftment after allogeneic bone marrow transplantation (BMT) [20]. It is also possible to detect somatic changes in human cancer cells and in blood cells of patients with hematological malignancies [24, 25, 28].

A 33-base pair (bp) minisatellite which was originally found in the first intron of the human myoglobin gene is capable of detecting other highly polymorphic minisatellites tandemly located in human chromosomes. These share the same common core sequence GGGCAGGAXG (X, A or G) preceded by a 5-bp sequence common to most but not all repeats. Minisatellite probe 33.15 $(AGAGGTGGGCAGGTGG)_{29}$, which has half of the repeat length of the 33-bp minisatellite but shares it core sequence, detects a complex profile of hybridizing fragments after *Hinf* I digestion of DNA. This hybridization pattern is highly polymorphic and the probability that two individuals have the same pattern is 3×10^{-11} [13]. Probe 33.6 $((AGGGCTGGAGG)_3)_{29}$ detects about 11 additional polymorphic fragments not detected by the 33.15. The probability that two individuals share all the fragments detected by 33.15 and 33.6 is 5×10^{-15}. Thus, the combined use of these two minisatellite probes provides highly individual hybridization patterns. In low stringency conditions the sequence GAGGGTGGXGGXTCT of M13 phage also detects polymorphic minisatellites in human DNA [30]. The hybridization pattern is different from that obtained with probes 33.6 and 33.15 of Jeffreys et al. [11-14] providing another set of individually specific DNA-F. After *Hinf* I or *Sau*3A digestion,

the ^{32}P-labeled minisatellite probes 33.15 + 33.6 and M13 detect 40–50 different bands depending on the individual. Under optimal conditions it is possible to detect extra or missing bands reliably, and only bands which are very close to intense original bands may remain "hidden".

The sensitivity of DNA-F analysis in the detection of minority DNA has been determined from DNA mixtures and also from DNA prepared from cell mixtures [20, 23, 34]. The detection limit has varied from 2% to 10%. The original intensity of the extra bands detected in pure blast cell populations is the most important factor determining the sensitivity of DNA-F analysis in the detection of minority DNA in remission (Fig. 1). The size of the fragments may also affect the detection limit since bigger fragments tend to hybridize more strongly with minisatellite probes than do smaller fragments [13]. The technical problems of DNA-F analysis have caused confusion in the court room and one should be cautious when interpreting inadequately controlled results [17].

DNA fingerprinting has been used in forensic medicine and for the identification of individuals in cases of disputed parentage [6, 10, 11]. We have studied one case where a male patient became a father several years post BMT. Since total body irradiation usually leads to infertility, the case was analyzed in detail; hybridizations with different polymorphic probes demonstrated unequivocally that the patient indeed was the father (S. Pakkala, in preparation). In syngeneic transplantations the confirmation of monozygosity is important because it obviates the need for immunosuppression and/or T-cell depletion of the graft, which is especially important when BMT is performed in advanced disease where T-cell depletion increases the risk of relapse post BMT. In twin transplantations where multiple transfusions invalidated the blood group and red cell isoenzyme analyses, syngeneity has been successfully demonstrated by DNA-F analysis [15].

Except for the identification of individuals, DNA-F analysis can be applied to the analysis of clonality in mixed cell populations. It is possible to demonstrate engraftment and characterize the origin of different cell populations or relapse after allogeneic BMT. Successful engraftment leads to total blood chimerism; engrafted hematopoietic cells are of donor origin and display a corresponding DNA-F pattern, which can be demonstrated 10 days post BMT [18, 20]. Mareni et al. [18] also compared DNA-F analysis with karyotyping. Usually very few mitoses are found shortly after BMT, but DNA-F analysis is informative in all cases which makes it suitable for follow-up studies. Leukemia relapse after BMT is preceded by mixed blood chimerism as demonstrated in 22 patients using DNA polymorphism in follow-up of T-cell-depleted BMT [3]. In 4 patients 10% of recipient-type DNA was detected before relapse and in 3 patients before graft failure, there were 6 patients still in remission with less than 5% mixed chimerism 9–31 months post BMT. It has been shown that in T-cell-depleted BMT the T-cells in relapse are of recipient origin. After appropriate fractionation it is also possible to study the origin of other bone marrow cell populations.

We have performed DNA-F analysis on 52 leukemia patients – 25 with acute myeloid leukemia (AML) and 27 with acute lymphoblastic leukemia (ALL) – and demonstrated that DNA-F of hematopoietic cells may be different in the blastic phase and in remission in about one-third of acute leukemia patients [24, 25]. The differences in DNA-F at diagnosis and in remission may be used as molecular markers of the disease. Hybridizations with 33.6 + 33.15 and M13 probes revealed DNA-F changes in

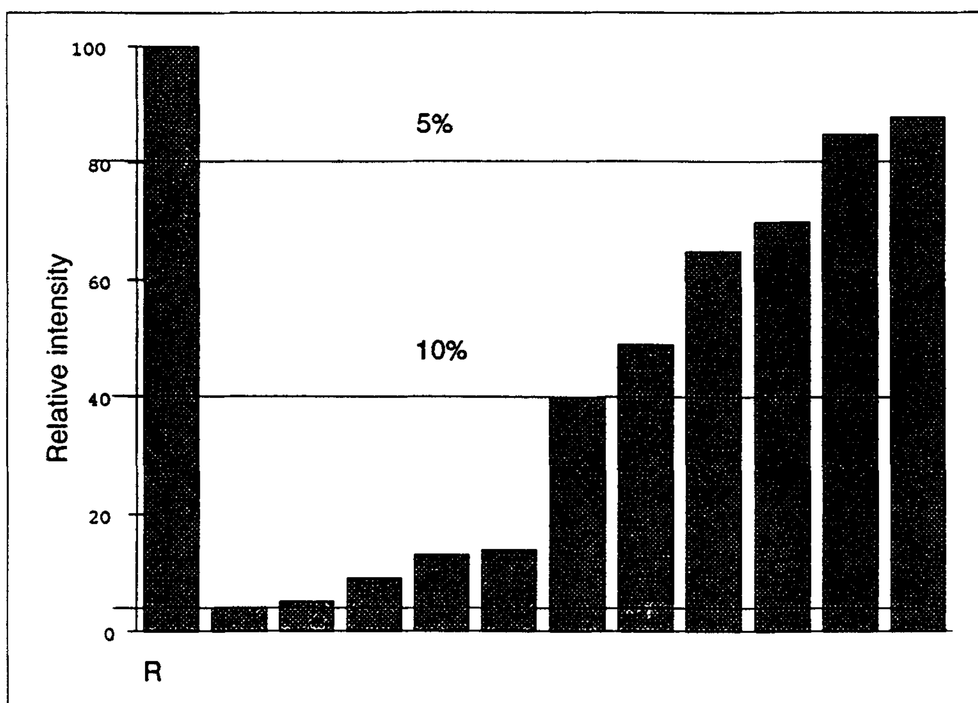

Fig. 1. Relative intensities of 11 extra bands detected by densitometry of DNA fingerprint (DNA-F) autoradiograph of leukemia cells. The two lines, 5% and 10%, indicate corresponding detection limits of DNA-F analysis: if the intensity of an extra band in a leukemic sample is 80, it can be detected in a remission sample with less than 5% of blast cells after density gradient centrifugation. *R*, Reference band with the relative intensity of 100

different patients: since the two probes produce different sets of restriction fragments [30], they may represent different areas of the genome. In 14 cases the DNA-F changes were detected only by one probe and in 5 cases DNA-F changes were detected by both probes. Thus, DNA-F analysis in leukemia patients should include hybridizations with both probes.

The individual sensitivity of the DNA-F method was demonstrated in 1 of our ALL patients, where DNA-F changes were detected weeks before other signs suggested relapse [24]. Routine follow-up is advised only if the extra DNA-F bands of the leukemia cells are intense enough to allow the detection of leukemia cells in a remission sample after density gradient centrifugation. The DNA-F analysis may be more useful in AML than in ALL where clonality can also be demonstrated by studies on immunoglobulin or T-cell receptor gene rearrangement.

The differences detected in DNA-F of leukemic cells may arise in several ways. The individual mutation rate to a new allele for these fragments is estimated to be about 10^{-3} [13]. It is not likely that spontaneous mutation is the cause of DNA-F changes because we detected single or multiple changes in one-third of the cases. *Hinf* I site terminating in methylated cytidine are resistant to enzymatic cleavage and differences in methylation of DNA may alter the *Hinf* I cleavage leading to changes in restriction fragments [22]. The DNA-F alterations are, however, not due to leukemia-specific methylation changes of DNA since *Alu* I digestion (not affected by methylation) also demonstrated corresponding DNA-F changes [9]. There might be alterations of restriction sites or unequal mitotic recombinations which become apparent due to the clonality. Chemotherapy may cause breaks in chromosomes and the differences in DNA-F could be due to therapy-related changes in DNA, but no alterations were found in 35 lymphoma patients studied before and after one to four courses of combination chemotherapy.

Combined with other techniques DNA-F analysis may assist in the preparation of new AML-specific probes. Although most chromosomes contain minisatellite loci, two major minisatellite loci have been found in chromosomes 1 (1q23) and 7 (7q35–36) [5], and a polymorphic DNA probe isolated from a human DNA-F has been used to demonstrate chromosomal 7 loss in myelodysplasia [29]. Whether the loci where DNA-F alterations have been found are important for the development of leukemia is currently not known. The comparison of DNA-F changes with karyotypic changes detected in leukemia cells has suggested a tentative association between minisatellite loci and chromosome 8 where two oncogenes (c-*mos*, c-*myc*) and an AML-related DNA sequence have been located [19, 21]. No specific translocations or deletions detected so far have altered DNA-F patterns suggesting that these areas are not related to minisatellites [26].

In summary, hypervariable regions of DNA may be used as markers of cell clones, either benign or malignant, and with chromosome-specific minisatellite probes it may be possible to reveal new DNA areas related to different diseases.

References

1. Auriol L d', Macintyre E, Galibert F, Sigaux F, In vitro amplification of T-cell gamma gene rearrangements: a new tool for the assessment of minimal residual disease in acute lymphoblastic leukemias. Leukemia 3:155, 1989
2. Botstein D, White RL, Skolnick M, Davis RW, Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am J Hum Genet 32:314, 1980
3. Bretagne S, Vidau M, Kuenz M, Cordonnier C, Henni G, Vinci G, Goossens M, Vernant JP, Mixed blood chimerism in T-cell depleted bone marrow transplant recipients: evaluation using DNA polymorphism. Blood 70:1692, 1987
4. Campana D, Yokota S, Coustan-Smith E, Hansen-Hagge TE, Janossy G, Bartram CR, The detection of residual acute lymphoblastic leukemia cells with immunologic methods and polymerase chain reaction: a comparative study. Leukemia 4:609, 1990
5. Chimini G, Mattei M-G, Passage E, Nguyen C, Boretto J, Mattei J-F, Jordan BR, In situ hybridization and pulsed field gel analysis define two major minisatellite loci: 1q23 for minisatellite 33.6 and 7q35–q36 for minisatellite 33.15. Genomics 5:316, 1989
6. Gill P, Jeffreys AJ, Werret DJ, Forensic application of DNA "fingerprints". Nature 318:577, 1985
7. Hagemeijer A, Adriaansen HJ, Bartram CR, In: Löwenberg B, Hagenbeck A (eds) Minimal residual disease in acute leukemia. Dordrecht, p. 1, 1986
8. Hansen-Hagge TE, Yokota S, Bartram CR, Detection of minimal residual disease in acute lymphoblastic leukemia by in vitro amplification of rearranged T-cell receptor delta chain sequences. Blood 74:1762, 1989
9. Helminen P, Does chemotherpy of haematological malignancies effect DNA fingerprint pattern. Leuk Res (in press)
10. Helminen P, Ehnholm C, Lokki M-L, Jeffreys A, Peltonen L, Application of DNA "fingerprints" to paternity determinations. Lancet I:574, 1988
11. Jeffreys AJ, Brookfield JFY, Semeonoff R, Positive identification of an immigration test-case using human DNA fingerprints. Nature 317:818, 1985
12. Jeffreys AJ, Wilson V, Thein SL, Individual-specific "fingerprints" of human DNA. Nature 316:76, 1985
13. Jeffreys AJ, Wilson V, Thein SL, Hypervariable "minisatellite" regions in human DNA. Nature 314:67, 1985
14. Jeffreys AJ, Royle NJ, Wilson V, Wong F, Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 332:278, 1988
15. Jones L, Thein L, Jeffreys AJ, Apperley JF, Catovsky D, Goldman JM, Identical twin transplantation for 5 patients with chronic myeloid leukaemia: role of DNA fingerprinting to confirm monozygosity in 3 cases. Eur J Haematol 39:144, 1987
16. Katz F, Ball L, Gibbons B, Chessells J, The use of DNA probes to monitor minimal residual disease in childhood acute lymphoblastic leukaemia. Br J Haematol 73:173, 1989
17. Lander ES, DNA fingerprinting on trial. Nature 339:501, 1989
18. Mareni C, Origone P, Sessarego M, Bacigalupo A, Frassoni F, Gualandi F, Ajmar F, Early and long term follow-up with minisatellite probes in bone marrow patients. Leukemia 4:704, 1990
19. Mars WM, Tuinen P van, Drabkin HA, White JW, Saunders GF, A myeloid related sequence that localizes to human chromosome 8q21.1–22. Blood 71:1713, 1988
20. Min GL, Hibbin J, Arthur C, Apperley J, Jeffreys A, Goldman J, Use of minisatellite DNA probes for recognition and characterization of relapse after allogeneic bone marrow transplantation. Br J Haematol 68:195, 1988
21. Neel BG, Jhanwar SC, Chaganti RSK, Hayward WS, Two human c-*onc* genes ar located on the long arm of chromosome 8. Proc Natl Acad Sci USA 79:7842, 1982
22. Nelson M, McClelland M, The effect of spite specific methylation on restriction-modification enzymes. Nucleic Acids Res [Suppl] 15:219, 1987

23. Pakkala S, DNA – fingerprinting in the detection of residual disease in acute leukemia. Leukemia 5:437, 1991
24. Pakkala S, Helminen P, Alitalo R, Saarinen U, Peltonen L, Differences in DNA-fingerprints between remission and relapse in childhood acute lymphoblastic leukemia. Leuk Res 12:757, 1988
25. Pakkala S, Helminen P, Ruutu T, Saarinen UM, Peltonen L, New molecular marker in AML: DNA – fingerprint differences between leukemic phase and remission in acute myeloid leukemia. Leuk Res 13:907, 1989
26. Pakkala S, Knuutila S, Helminen P, Ruutu T, Saarinen UM, Peltonen L, DNA – fingerprint changes compared to karyotypes in acute leukemia. Leukemia 4:866, 1990
27. Radich JP, Kopecky KJ, Willman CL, Weick J, Head D, Appelbaum F, Collins S, N-*ras* mutations in adult de novo acute myelogenous leukemia: prevalence and clinical significance. Blood 76:80, 1990
28. Thein SL, Jeffreys AJ, Gooi HC, Cotter F, Flint J, O'Connor NTJ, Weatherall DJ, Wainscoat JS, Detection of somatic changes in human cancer DNA by DNA fingerprint analysis. Br J Cancer 55:353, 1987
29. Thein SL, Oscier DG, Jeffreys AJ, Hesketh C, Pilkington S, Summers C, Fitchett M, Wainscoat JS, Detection of chromosomal 7 loss in myeloidysplasia using an extremely polymorphic DNA probe. Br J Cancer 57:131, 1988
30. Vassart G, Georges M, Monsieur R, Brocas H, Lequarre AS, Christophe D, A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science 235:683, 1987
31. Weil SC, Rosner GL, Reid MS, Chisholm RL, Lemons RS, Swanson MS, Carrino JJ, Diaz MO, Le Beau MM, Translocation and rearrangement of myeloperoxidase gene in acute promyelocytic leukemia. Science 240:790, 1988
32. Wessman M, Ruutu T, Volin L, Knuutila S, In situ hybridization using a Y-specific probe – a sensitive method for distinguishing residual recipient cells from female donor cells in bone marrow transplantation. Bone Marrow Transplant 4:283, 1989
33. Yamada M, Hudson S, Tournay O, Bittenbender S, Shane SS, Lange B, Tsujimoto Y, Caton AJ, Rovera G, Detection of minimal disease in hematopoietic malignancies of the B-cell lineage by using third-complementarity-determining region (CDR-III)-specific probes. Proc Natl Acad Sci USA 86:5123, 1989
34. Yoffe G, Blick M, Kantarjian H, Spitzer G, Gutterman J, Talpaz M, Molecular analysis of interferon-induced suppression of Philadelphia chromosome in patients with chronic myeloid leukemia. Blood 69:961, 1987