High resolution comparative genomic hybridization detects 7–8 megabasepair deletion in PCR amplified DNA [1]

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We investigated if any change in spatial resolution of comparative genomic hybridization analysis could be detected when using DNA amplified by degenerate oligonucleotide primed PCR (DOP-PCR) as opposed to the use of unamplified DNA. Five DNA samples from B-cell leukemias with small 11q deletions were amplified by DOP-PCR and analysed by means of high resolution comparative genomic hybridization (HR-CGH) for the evaluation of aberration size detection limit. By means of HR-CGH, we found the detection limit of DOP-PCR CGH for deletions to be between 3 Mbp and 7–8 Mbp.

Keywords: High resolution comparative genomic hybridization, DOP-PCR, B-cell leukemia

1. Introduction

CGH is a technique used for the comprehensive detection of chromosomal segments with copy number imbalances in a single experiment [2]. This has made CGH a powerful tool for cytogenetic analysis. As a routine CGH experiment requires 1 μg test DNA, DOP-PCR for amplification of test DNA is often used as a prerequisite for CGH analysis of microdissected or flow sorted cell populations [5,8,10].

Previous attempts to investigate the spatial limit for detection of aberrations with CGH include a theoretical study based on assumptions about DNA condensation in chromosomes, hybridization noise, and image formation in camera and microscope under standard conditions. The detection limit depending on the number of copies that were gained or lost, a theoretical lower limit were 2 Mbp for loss of 1 copy and 1 Mbp for loss of 2 copies [7].

The detection limit of CGH has also been estimated experimentally by analysis of a series of samples with consecutively diminishing deletions on chromosome 11, these experiments showed the detection limit to be between 10 and 3 Mbp depending on the protocol in use [1,3]. However, the effect of the DOP-PCR amplification step on the resolution of CGH analysis remains to be elucidated.

2. Materials and methods

2.1. Material

Analyses of a series of B-cell leukemias using FISH have mapped the size of deletions on chromosome 11q and the fraction of cells carrying the deletion [9]. DNA samples from 5 of these leukemias with deletion sizes ranging from 3 Mbp to 14–18 Mbp have previously been analyzed in two CGH studies for testing the spatial resolution of CGH, using either fixed threshold levels [1] or HR-CGH [3]. DNA from these 5 samples was DOP-PCR amplified and fluorescein labelled using the following procedure.

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2.2. DNA amplification

The samples were diluted to 1 ng/μl in distilled water and stored frozen. Three nanogram DNA template was added to a total volume of 30 μl reaction solution: 10 mM Tris-HCl, 50 mM KCl, 1.8 mM $MgCl_2$, 0.01% Triton X-100, 0.005% vol/vol gelatine, 250 μM dNTP, 1 μM UN-1 primer (5′-CCG ACT CGA GNN NNN NAT GTG G-3′) (TAG Copenhagen A/S, Copenhagen, Denmark), 0.166 U/μl Taq DNA polymerase (Amplitaq LD, PE Biosystems, Foster City, CA, USA), pH 8.3. Buffer, nucleotides and primers were filtrated through a 0.2 μm filter (Acrodisc, Pall Gelman, Ann Arbor, MI, USA).

The cycling conditions consisted of 5 low-stringency cycles: 30 sec denaturation (94°C), 60 sec annealing (25°C), and 120 sec elongation (72°C); followed by 35 high-stringency cycles: 20 sec denaturation (94°C), 30 sec annealing (56°C), and 120 sec elongation (72°C). The products from two PCR reactions were pooled and the DNA was precipitated. The pellet was dried and redissolved in 15 μl sterile water [5].

2.3. Comparative genomic hybridization

The test DNA was labelled directly with fluorescein-dUTP, by means of standard nick translation reaction. As DOP-PCR amplification gave DNA fragments with a short length of 1–4 kilobases, the reaction was modified in order to obviate a further reduction of DNA fragment size. In brief: The DNA was labelled in 48.3 μl labelling buffer consisting of 0.02 mM dATP, 0.02 mM dCTP, 0.02 mM dGTP, 20 mM fluorescein-dUTP (DuPont, Boston, MA, USA), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, and 50 mM Tris-HCl at pH 7.8. The reaction was catalyzed by 1 μl DNA polymerase I (10 U/μl, Promega, Madison, WI, USA) and 0.75 μl enzyme mix, consisting of 500 U/ml DNA polymerase I, 1 U/ml DNAse I, 100 μg/ml BSA, 5 mM magnesium acetate, 1 mM 2-mercaptoethanol, 50 mM Tris-HCl at pH 7.5, 50% (vol/vol) glycerol. The mixture was incubated at 15°C for 40 minutes and the reaction was stopped by heat inactivation.

The reference DNA was prepared by direct Texas Red labelling of unamplified genomic DNA using nick translation under standard circumstances. Test DNA was mixed with 400 ng reference DNA and 20 μg Cot-1 DNA and hybridized to normal human metaphases at 37°C for 4 days. Image acquisition and analysis were performed as described previously [4].

Table 1
Sensitivity in detection of 11q deletions according to CGH methodology

Case No.	Approximate size of deletion (Mbp)[a]	Frequency of cells with deletion (%)[a]	HR-CGH, unamplified DNA[b]	HR-CGH, DOP-PCR amplified DNA
1	14–18	84	Yes	Yes
2	10–12	88	Yes	Yes
3	10–12	54	Yes	Yes
4	7–8	55	Yes	Yes
5	3	82	Yes	No

[a]Previously published results [9].

[b]Previously published results [3].

2.4. Evaluation

HR-CGH is a concept to evaluate CGH ratios by comparison to dynamic standard reference intervals as opposed to the use of fixed ratio limits. In certain chromosomal areas CGH analysis of normal test- and reference DNA will result in reproducible characteristic deviations from the expected ratio value of 1.0. By combining the profiles from several normal/normal hybridizations, a standard reference interval can be produced. The CGH profiles are evaluated by comparison of the 99.5% confidence interval of the mean sample profile ratio and the standard reference interval. Where no overlap between the intervals is seen the area is judged as aberrant [4]. The pattern of non-specific deviations in DOP-PCR CGH differs from the pattern found in CGH with unamplified DNA. Thus a specially designed dynamic standard reference interval was produced by performing a series of normal/normal DOP-PCR CGH experiments.

The CGH experiments on the B-cell leukemias were repeated several times in order to achieve optimal results regarding a series of quality requirements: signal-to-noise ratio, standard deviation of profiles, degree of suppression of intersperced repetetive sequences, background level, differences of homologue chromosomes. These quality requirements formed the basis for the selection of the analysis for the “yes/no” answer in Table 1. Which deletion size corresponded to each of the 5 samples was not known to the analysts until completion of all analyses.

3. Results

Deletions were detected in 4 of the 5 leukemias as compared to 5 in 5 using HR-CGH on unampli-

fied DNA (see Table 1). No false positive aberrations were detected. A good concordance was demonstrated between the length of the deletions determined with FISH, with HR-CGH using unamplified DNA, and with HR-CGH using DOP-PCR amplified DNA (see Fig. 1). However, HR-CGH using DOP-PCR amplified DNA resulted in a slightly decreased resolution compared to HR-CGH using unamplified DNA [3].

4. Discussion

The reduced sensitivity of HR-CGH using DOP-PCR amplified DNA can be ascribed to several causes. The nick translation reaction is adjusted to produce DNA fragments with minimally reduced length, this may result in less efficient labelling compared to routine labelling of genomic DNA. The secondary structure of the amplified DNA may be less suited for nick translation labelling or hybridization. The amplified DNA may contain an overrepresentation of repetitive sequences due to non-specific self-priming during the amplification procedure. The dynamic standard reference interval is comprised of selected cases from a far smaller database of normal/normal hybridizations than the dynamic standard reference interval for unamplified DNA. This increases the risk for the dynamic standard reference interval generated from DOP-PCR amplified material not being entirely representable, which could be a cause for the decreased sensitivity. On the other hand, in cases No. 3 and 4 where only 54–55% of the test DNA represents the aberrant genotype, the observed CGH resolution should be considered as underestimated, due to the weakening of the CGH signals by the dilution of test DNA with normal cell DNA [6].

In conclusion, the technique of HR-CGH is applicable to DOP-PCR amplified DNA, and the resolution is enhanced as compared to CGH evaluated with fixed ratio limits, and close to the resolution obtained with HR-CGH using unamplified DNA.

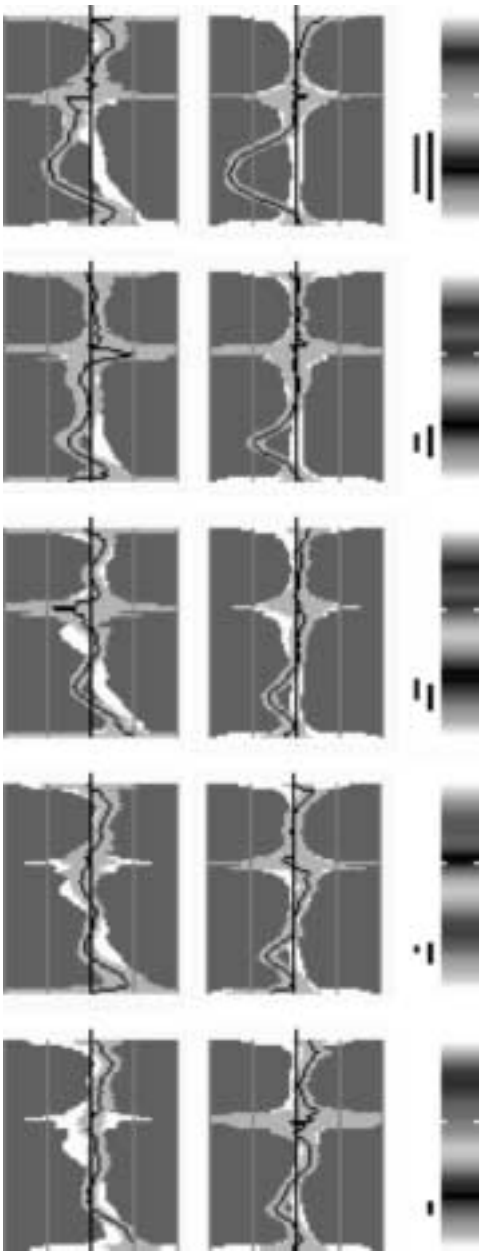

Fig. 1. Chromosome 11 from each analysis of the five cases of leukemia. The left column represents HR-CGH using DOP-PCR amplified DNA, and the right column represents HR-CGH using unamplified DNA. The mean ratio profile is shown in black along with the 99.5% confidence interval superimposed on the standard reference interval shown in white. The vertical lines represent the ratio values 0.5, 0.75, 1.0 (black), 1.25, and 1.5. To the right are shown the mean inverted DAPI chromosomes. The bar closest to the mean inverted DAPI chromosome represents the deletion detected by HR-CGH using unamplified DNA [3], and the bar farthest away the deletion detected by HR-CGH using DOP-PCR amplified DNA. The cases are from top to bottom: case 1 (14–18 Mbp), case 2 (10–12 Mbp), case 3 (10–12 Mbp), case 4 (7–8 Mbp), case 5 (3 Mbp).

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