Fluorescence *in situ* hybridization (FISH)

• Sample (tissue/cell culture/chromosome) preparation

Bone marrow smear pretreatment

Five fresh bone marrow smears were taken from thepatient, and the slides were baked at 56˚C for 10-20 min after natural drying. Room temperature 100% methanol solution and 100% glacial acetic acid solution were used for fixation for for 5 and 30 min, respectively. The glass slides were placed at -20˚C for precooling and gradient dehydration with ethanol for 2 min each.

Chromosome preparation and karyotype analysis:

Chromosome analysis using GTG banding was performed according to standard procedure (1). A total of 20 metaphase cells derived from the unstimulated bone marrow of the patient were analyzed. Karyotypes were described according to the international system for human cytogenetic nomenclature (2). For each patient, 20 metaphase dividing cells were analyzed as far as possible, and the karyotype of each specimen was identified by at least two physicians. The remaining cell suspension was stored at -20˚C for D-FISH detection.

• Operational steps of FISH

Specimen preparation and denaturation: The slides were soaked in denaturation solution for 5 min and then sequentially placed in precooled ethanol for gradient dehydration and air drying. Slides were preheated for 2-5 min in a baking machine at 45-50˚C and hybridized with the probe.

Preparation and denaturation of probe mixture: At room temperature, 7 μl hybridization buffer, 1 μl deionized water and 2 μl probe were put in a water bath box at 73.1˚C for denaturation for 5 min. The test tube was placed in a water bath box at 45-50˚C and taken out before hybridization.

Hybridization of probe and sample: A total of 10 μl of denatured probe mixture was dropped on a glass slide for hybridization. The slides were placed in a preheated wet box and hybridized overnight in an incubator at 42˚C.

Slide washing: The cover glass was removed from the hybridized slide, put into 0.4X SSC/0.3% NP-40 solution preheated at 67˚C, washed for 30 sec and dried at room temperature.

Double dyeing: A total of 15 μl DAPI was added and a cover glass, and double dyeing was performed for 20 min.

• Name, manufacturer, catalogue number, conjugate and dilution of primary and secondary antibodies, as well as temperature/duration of incubation

The immunohistochemical tests were performed in Carnoy fixed cell suspension as previously described (4). A rabbit polyclonal CENP-B antibody (Abcam) was used to stain all centromeres. The specific staining of the active centromeres was performed with the anti-CENP-C antibody. FITC-labeled goat anti-rabbit IgG and CyTM3-conjugated AffiniPure goat anti-guinea pig IgG (Dianova GmbH; Biozol) were applied as secondary antibodies.

• Method of detection, including type of microscope, wavelength, magnification.

FISH detection:

FISH was performed using the two-color dual-fusion DNA probe provided by Beijing Jinpujia Company, according to the manufacturer's protocol and a previous study (4). Finally, 500 cells were counted under the OLYMPUS-BX 51 fluorescence microscope (Olympus Corp.). The normal cell signal display was 2 red, 2 green, and the BCR/ABL fusion gene-positive cells were 2 red, 1 green, 1 yellow, or 1 red, 1 green, 1 yellow.

Software used for analysis.

Data analysis was performed with a standard software Affymetrix Genotyping Console™. For statistical analysis, GraphPad Prism software (Dotmatics) was used.

•Probes.

FISH and BCR-ABL

Oligonucleotide primer set:

According to the principles of PCR primer design, and referring to the relevant literature reports (6), seven nested primers and internal control primers were designed in total (Fig. 1), where E and D are ABL internal control primers, and the rest were used to amplify BCR/ABL mRNA. All oligonucleotide primers were synthesized by the Shanghai Cell Biotech Research Center.

The base order of each oligonucleotide was as follows:

A; 5'-GGAGCTGCAGATGCTGACCAAC-3'
B: 5'-TCAGACCCTGAGGC'TCAAAGTC-3'
C: 5'-GCAGCAGAAGAAGTGTTTCAG-3'
D: 5'-GTGATTATAGCCTAAGACCCG-3'
E: 5'-ACCATCGTGGGCGTCCGCAAGA-3'

Preparation of the RNA:

Ficoll peripheral blood mononuclear cells and K562 cell lines were isolated. Cells were lysed in RNA extraction buffer containing 0.5% Np 40, nucleated by centrifugation, phenol, chloroform, isopentanol extraction and ethanol precipitated RNA. After separating cells from most specimens, 100 to 200 μl of 0.1% DEPC aqueous solution was added directly and boiled in boiling water for 8 min and centrifuged with 10 to 20 μl of the supernatant for cDNA synthesis (7). The steps were performed in sterile conditions and all the items used were treated with DEPC.

Reverse recording reaction (RT):

The RT was modified according to the Hernardez (8) method. In the 20 μ1 total reaction solution, containing 40 units 0.1 mol/l RNasin, 2 μ1 DTT, 200 units, 2 mmol/l M-MIV reverse transcriptase and 5 μ1 20 pmol/1 dNTP ABL lateral primers D, PCR was performed at 37˚C for 50 min.

First step of the PCR reaction:

In 30 μl total reaction solution containing RT and l0 μl, 1.5 units Taq DNA polyenzyme, 20 pmol/l BCR lateral primer C or F and an appropriate amount of PCR reaction buffer, PCR was performed for 30 cycles, including 95˚C denaturation, 60˚C annealing and 73˚C extension for 1 min each.

The second step of PCR:

The first PCR product was added to the second step PCR reaction solution, with the medial nested primers A, B or G, B and Tag DNA polyenzymes, and the first step PCR conditions.

Detection of the PCR products:

A total of 8 μl secondary PCR products were analyzed using 8% polyacrylamide gel electrophoresis, ethidium bromide staining, ultraviolet fluorescence development and photography.

References

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