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Use of the Primed in situ Labelling(PRINS) for Chromosome 13 and Y

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Introduction

The majority of aneuploidies in newborns involve chromosomes 13, 18, 21, X and Y.

Aneuploidies involving these 5 chromosomes can account for up to 95% of all liveborn chromosomal abnormalities(Hook et al., 1985) and 67% of all chromosomal abnormalities if balanced translocations are included, whereas trisomy 16 and 45,X are the most frequent aberrations seen in abortuses (Lauritsen, 1982). Up to now, karyotyping remains the most conclusive test for chromosome identification, but there is a real need for a rapid method for detection of the major aneuploidies, especially in prenatal diagnosis where rapid results are desired.

Fluorescence in situ hybridization(FISH) has provided a direct way to detect DNA sequences(Pinkel et al., 1988 ; Trask, 1991) and numerous chromosome analyses by FISH have been represented (Nederlof et al., 1990 ; Ried et al., 1992), suggesting that this could be a reliable technique for detecting chromosomal abnormalities(Cremer et al., 1988).

The currently available DNA probes are α-satellite repeat probes and there are no repetitive probes specific for chromosome 13 and 21(Zahed et al., 1992). Production of locus-specific probes is laborious and time-consuming, and the procedure is not accessible for all cytogenetic laboratories. The primed in situ labelling(PRINS) technique(Koch et al., 1989, 1991) provides another way for the direct detection of the human chromosome and has been steadily improved in both sensitivity and versatility(Gosden et al., 1991 ; Gosden & Hanratty, 1993 ; Gosden & Lawson, 1994a ; Hindkjaer et al., 1994). It is based on the annealing of specific oligonucleotide primers and subsequent primer extension by Taq DNA polymerase in the presence of labelled nucleotides. I present the appplication of this protocol to the rapid detection of chromosome 13 and Y in interphase nuclei:

Materials and Method

Selection and preparation of primers

Alpha-satellite DNA sequences have been described for all human chromosome. Each α-satellite sequence was compared to the consensus α-satellite sequence of human chromosome reported by Choo et al.(1991). All oligonucletide primers were synthesized on a Applied Biosystems Model 381A according to the manufacturers instructions.

The sequences of Yc and 13A were 5CTTGGAGACCTTTGTGGAAG3(56℃ annealing temperature) and 5TGATGTGTGTACCCAGCT3(60℃ annealing temperature) each other.

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Slide preparation

Interphase cells were prepared from cord blood using standard cytogenetic procedure. For optimal results, slides were passed through an alcohol series(70 %, 90 % and 100 %) at room temperature for 2 min each other, which helped in preserving chromosome morphology, and air-dried before the first PRINS reaction.

Slides were denaturated by immersion in 70 % formamide-2X SSC at 70℃ for 3 min(2 min) and then immediately dehydrated in a series of 4℃ ice -cold ethanol washes 70-90-100 % for 2 min, each other before being allowed to air-dry.

PRINS reaction

For each slide, the reaction mixture was prepared in a final volume of 50 ul reacton mix of Taq buffer, containing 50~250 ng of one primer of primer pair(100~300 pmol of the oligonucleotide), 0.2 mM each of dATP, dCTP, dGTP, 0.02 mM dTTP, 0.02 nmM of either biotin-16-dUTP(Boehringer) or digoxigenin-11-dUTP(Boehringer) or fluorescin(FluoroRed or FluoroGreen)-12-dUTP(Amersham), 50 mM KCL, 10 mM TRIS-HCL. pH 8.3, 1.5 mM $MgCL_2$, 0.01 % bovine serum albumin(BSA) and 2~2.5 U(1 U)of Taq DNA polymerase(Cetus or Boehringer). This mixture were placed under the cleaned coverslip on the slides and sealed with rubber cement.

The procedure was performed on a programmable temperature cycler Techne PHC-3, fitted with a flat plate block. The programme consisted of 1 5 min at the annealing temperature(according to the primer used) and then, 30 min at 72℃. Thus, chromosome slides, coverslips and reaction mixtures were incubated at the selected annealing temperature for 5 min. Then, 50 ml of each mixture was deposited on a slide, spread with a coverslip and slides were heated for a further 10 min. At the beginning of the second step, the temperature was automatically raised to 72℃ and slides were incubated 30 min in order to allow the nucleotide chain elongation.

The reaction was stopped by immersing the slides in 500 mM NaCl, 59 mM EDTA, pH 8 at 72 ℃ for 5 min. The slides were then transferred to 4xSSC, 0.2 % Tween 20 at room temperature, and could be left in this solution for several hours for convenience.

Microscopic analysis

Detection of the labelling site was performed by immunocytochemistry and conventional fluorescence microscopy. Biotinylated fragments were visualized using fluoresceinated avidin(5 mg/ml), whereas detection of digoxigenin incorporated into the synthesized product was done with anti-digoxigenin-labelled antibody(20 mg/ml). Alternatively, DNA fragments labelled with fluorescein were directly visualized. The preparations were counterstained with DAPI and examined with a Zeiss epifluorescence microscope. Photomicrographs were taken at ×500 magnification of Kodak Ektachrome 400 ASA film.

Results

PRINS reaction were performed on several cord blood samples. Under the defined conditions, specific chromosomal labelling was obtained on interphase nuclei with each primer tested. All the primers provided strong and well defined signals on interphase nuclei(Fig. 1, 2).

In order to assess the efficiency of the method, various practical condition and tests will be needed.

Discussion

Primed in situ(PRINS) labelling has become an alternative to in situ hybridization(ISH) for the localization of nucleic acid sequences in cells and tissue .

Chromosomal abnormalities are currently diagnosed by cytogenetic analysis of metaphase chromosome. The ability to detect multiple targets rapi-

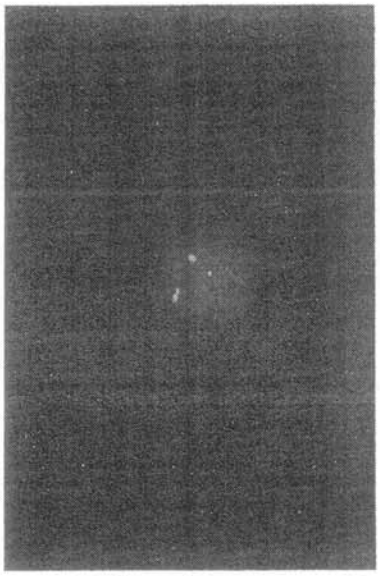

Fig. 1. Specific labelling of chromosome 13(red) and Y(green) on interphase cells.

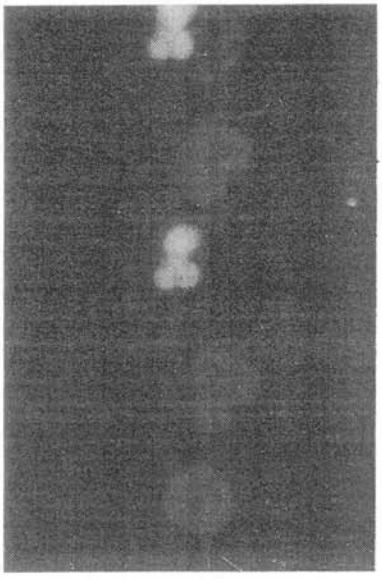

Fig. 2. Specific labelling of chromosome 13(red).

dly by sequential PRINS reaction is an important, forward step for detecting the common abnormalities. Most of the available probes for FISH are centromeric probes repeat probe, but there are no specific centromeric probes for chromosome 13 and 21, because of the high homology of their centromeric sequence(Ried et al., 1992). Specific labelling for locus-specific probe required, but is still laborious and not accessible to all laboratories.

For the PRINS reaction, there are advantages of the high specificity between primers and their genomic targets to determine a set of primers specific for individual chromosome.

The lack of stringency of primer annealing and the inability of some Taq polymerase accurately to perform the elongation reaction are the weakness of the PRINS(Gosden & Hanratty, 1991), but the use of programmable thermocycler results in an optimization of both annealing and extension condition. As in FISH, both the cell quality and the slide preparation are important factors for the success of the reaction. The use of short oligonucleotides(19~27 mers), at concentrations of only 50~250 ng per slide, proved to be sufficient to produce intense, specific and distinct staining of chromosomal DNA sequence. Although the primer oligonucleotides used in the sequential reactions may differ in their optimal annealing temperature, this dose not affect the outcome of the reaction, as the labeled product produced in situ has much greater thermal stability than oligonucleotide, and is retained even at high temperature(Gosden & Hanratty, 1993)

In conclusion, the PRINS technique provides an interesting alternative the in situ hybridization and may be a reliable technique for detecting aneuploidies and some chromosome aberration.

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=국문초록=

13, Y 염색체진단의 Primed in situ Labelling (PRINS)의 이용

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염색체 이상의 대부분은 13, 18, 21상염색체와 X,Y성염색체의 이상에 기인하며 이들 염색체 이상의 진단은 배양에 의한 염색체 분석에 의해 이루어 짐으로, 이들 염색체 이상의 빠른 결과를 기대하여, Fluorescence in situ hybridization(FISH)등의 진단법이 이용되고 있다. 그러나 대부분의 probes는 alpha-satellite probe이며, 염색체 13번과 21번의 특이 probes는 사용에 매우 어려움이 있다. PRINS는 세포나 조직의 핵산 배열의 위치에 사용되는 in situ hybridization의 한 방법으로서 특이한 oligonucleotide primer의 annealing과 labeled nucleotide의 존재하에 Taq polymerase의 primer extension을 통한 염색체이상을 진단하는 방법으로서, 본 연구는 염색체 13,Y의 이상 진단을 위하여 제대혈의 간기내 세포와 염색체 13,Y의 specific primer를 이용하여 염색체 13, Y의 labelling을 확인함으로서, PRINS를 이

용한 진단법이 in situ hybridization의 다른 방법으로서의 확립과 염색체 이수 배수성과 다른 염색체 이상의 산전 진단에 도움이 되며, 또한 여러 가지 염색체 이상의 동시 진단이 가능하리라 사료됩니다.

Key Words : **PRINS, FISH,** 염색체 이수 배수성.