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Three-color Fluorescence in Situ Hybridization (FISH) Using Chorionic Villi Sampling(CVS) Transport Media

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I. Introduction

The major aneuploidies diagnosed prenatally involve the autosome 13, 18, and 21, and sex chromosome(Whiteman & Klinger, 1991). Actually prenatal diagnois of these aneuploidies is routinely accomplished by full karyotype analysis after amniocentesis or CVS(chorionic villi sampling). This technique allows the detection of a broad range of numerical and structural aberrations but it is laborintensive, time consuming, and requires highly trained analysts. Thus, prenatal screening for chromosome aberrations is performed only for pregnant women considered to be at risk. FISH(fluorescence in situ hybridization) allows the rapid analysis of chromosome copy number in interphase cells using chromosome specific probes(Cremer et al., 1986 ; Julien et al., 1986 ; Lichter et al., 1988 ; Pinkel et al., 1988). Fetal cells exist in maternal blood and can be used for prenatal diagnosis. Prenatal diagnosis using fetal cells isolated from maternal blood now seems a real possibility(Simpson & Elias, 1993). For the study of isolation of fetal cells in maternal blood, peripheral blood is obtained from pregnant women prior to invasive procedures, either CVS or amniocentesis. In the absence of a reliable fetal cell specific marker, the sensitivity of fetal cell detection is limited to the presence of either a positive signal for the Y chromosome or fetal trisomy. Therefore, in the development of protocols for isolation and analysis of fetal cells from maternal blood, studies must conduct on pregnant women carrying males fetuses. In maternal blood samples obtained prior to CVS, rapid determ ination of fetal sex is necessary for immediate and efficient processing of those maternal blood samples used in assessing various experimental parameters. I report here prospective rapid FISH for identification of fetal sex using CVS transport media.

II. Materials and Methods

Chorionic villus samples

Transabdominal and transcervical CVS from 7 ~10 week pregnancies were performed for prenatal diagnosis from 20 women of advanced maternal age and ultrasound fetal anomalies. Samples contained more than 35 mg of tissue. Tissue from every sample was used for cell culture and subsequent conventional chromosomal analysis. And the remaining CVS(500 ul in transport media) was used for direct in situ hybridization analysis.

Slide preparation

Uncultured CVS in 500 ul transport fluid for FI-

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SH were centrifuged at 18,000 rpm for 5 minute after directly add 100ul of Carnoys solution(3 : 1 methanol : glacial acetic acid). Then resuspend pellet in 100~200 ul of fresh Carnoys solution, then centrifuge to collect cells. Places 50 ul of cell suspensi on on clean slide after centrifuge, and complete air dry.

Fluorescent in situ hybridization(FISH)

Dehydrate slide with series of EtOH washes for 2 minutes each and air dry. The 10 ul probe mix of CEM buffer, 0.5 ul XY probe(Vysis, Inc), and 0.5ul 18 probe(Vysis, Inc). Place the probe mixtures on slide and sealed with rubber cement, and allowed glue to complete air dry before transferring the slide to the flat bed of a HYBRID Omnigene thermal cycler for denaturation at 80℃ for 1 minute, then allow the slide to hybridize for 30 minutes in humidity chamber at 37℃.

Post washing and detection

Place the slide in 0.25 X SSC at 68℃ for 2 minutes 30 seconds. After three 2 minutes washes in 1 X PBD, the nuclei were counterstained with DAPI and reviewed using a Zeiss Axioskop with triple band pass filter that allows simultaneous visualization of green, red and yellow signals on a blue (DAPI) background.

DNA probes

I used X(directly labeled in Spectrum Red) and Y(directly labeled in Spectrum Green), and 18(directly labeled in Spectrum Yellow) probes as a control probe were use simultaneously(Vysis, Inc.).

III. Results

A total of 20 CVS samples(10 males and 10 females) were obtained for determination of fetal sex and were analysed by FISH and full karyotype.

Fetal sex can be determined within two hours of the CVS procedure. Chorionic villi are not required for this procedure, therefore, cytogenetic results are not compromised. This procedure may be helpful in cases of failed CVS culturing. Approximately 200 nuclei were scored per slide. Overall,

Table 1. Detection of frequency using alpha-satelliye probe for X,Y, and 18

Fetal Sex-	No. of cases correctly Indentified by Karyotype	Mean % of cells with one Y signal and two 18 signal
Male	10/10(100 %)	32 %(5.4 %~64 %)
Female	10/10(100 %)	0 %

Table 2. No. of male, female diagnosed by FISH and full karyotype among 20 samples

Sex chromosome constitution	FISH	Full karyotyping
XX	10	10
XY	10	10

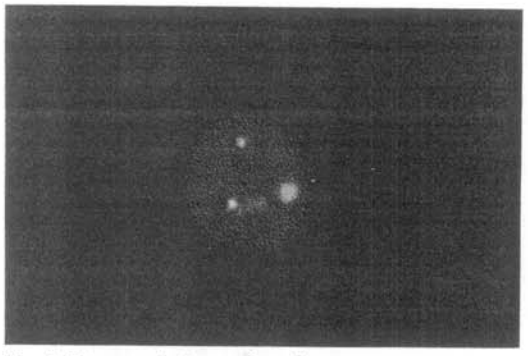

Fig. 1. Representative male cell in uncultured.

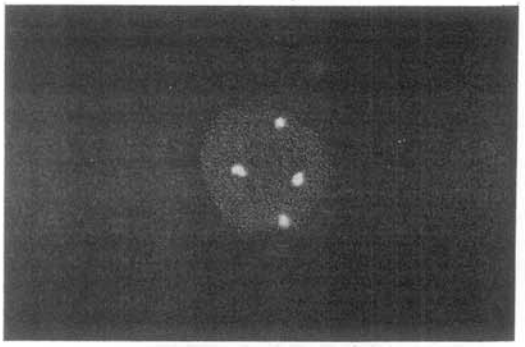

Fig. 2. Representative cell with two XX CVS with one X(red) and one Y(green) signal signals(red) in uncultured CVS with two with two 18 signals (yellow).

fetal sex was correctly identified in 10/10(100 %) male cases and female cases and a range of 5.4 % to 64 %(average 32 %). Yinterphase cells signal were detected in 10 male cases, but not detected Y interphase signal in 10 female cases (Table 1, 2 ; Fig. 1, 2). Total time to complete procedure and analysis is under two hours.

Ⅳ. Discussion

I have presented the evaluation of commercially available alpha satellite DNA probes for prenatal diagnosis in uncultured chorionic villi sampling(CVS) and have demonstrated that X,Y and 18 chromosome specific DNA probes can be used for the detection of fetal sex and aneuploidies in interphase nuclei from uncultured CVS. Fluorescence in situ hybridization is new technology with the potential for quickly identifying chromosomal abnormalities. Originally a research tool, it has rapidly achieved a role in prenatal, cancer, and infectious disease diagnosis and dosimetry. If we are to consider the role of FISH in uncultured chorionic villi sampling(CVS) as a non-invasive diagnostic test either independent of or in combination with other markers(eg. hCG, uE3, and MSAFP) for fetal aneuploidy, we must determine the level of sensitivity that is achievable by this approach. FISH analysis of chromosome X,Y,13,18, and 21 would for about 95 % of chromosomal abnormalities demanding serious medical attention. But FISH on interphase nuclei is limited by 1) availability and specificity of chromosome-specific probes ; 2) availability of fluochrome and/or fluophore labeled probes with spectrally well separated emissions ; 3) availability of excitation and emission filters for the visualization of the primary and mixed colors ; 4) difficulties in counting an/or spatial relation of hybridization signals. This was a preliminary trial with a small sample set. Further clinical evaluations will be required to establish proper diagnostic protocols and criteria In the future, detection of fetal sex and trisomy by FISH may become a rapid means of screening the uncultured chorionic villus samples from pregnant women at high risk for fetal aneuploidy and this method. allows for effiital selection of samples for protocol development in the isolation and detection of fetal cells from maternal blood.

Ⅴ. Conclusion

I have presented the evaluation of commercially available alpha satellite DNA probes for prenatal diagnosis in uncultured chorionic villi sampling(CVS) and have demonstrated that X,Y and 18 centromeric DNA probes can be used for the detection of fetal sex and aneuploidies in interphase nuclei from uncultured CVS. Fetal sex can be determined within two hours of the CVS procedure. Chorionic villi are not required for this procedure, therefore, cytogenetic results are not compromised. This procedure may be helpful in cases of failed CVS culturing. This was a preliminary trial with a small sample set. Further clinical evaluations will be required to establish proper diagnostic protocols and criteria. In the future, detection of fetal sex and trisomy by FISH may become a rapid means of screening the uncultured chorionic villi samples from pregnant women at high risk for fetal aneuploidy and this method.

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=국문초록=

융모막조직을 이용한 산전진단에서 형광결합보체법 (Fluorescence in Situ Hybridization)의 이용

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임신 중 진단되는 대부분의 이수 배수체의 이상은 상염색체 13, 18, 21과 성염색체의 이상으로 이루어지며, 이 이수 배수체의 이상유무는 양수검사 혹은 융모막검사 등을 통한 일반적인 염색체분석에 의해 진단되어 진다. Fluorescence in situ hybridization(FISH)은 간기 세포 내의 염색체의 수를 빠르게 분석하게 하는 방법으로 많이 연구되고 있다. 또한 양수 천자, 융모막검사 등의 침윤성 검사에 의한 염색체이상의 분석 이전에 모체혈 내의 태아혈을 이용한 산전진단법이 연구되고 있으나, 모성의 세포와 태아의 세포의 구분에 많은 어려움이 있어, 침윤성검사 전에 채취한 모성혈 및 융모막조직을 이용하여, Fluorescence in situ hybridization(FISH) 을 이용한 태아의 성의 감별, 태아 이수성 유무의 감별은 모체혈의 산전진단에의 이용에 필요한 많은 실험적 지표에 상당한 유용성의 가치가 있을 것으로 사려되어, 본 연구를 시행하였다. 검사채취로부터 2시간 내의 빠르고도 정확한 결과를 구하였으나 ,향후 정확한 산전진단에의 적용을 위해서는 검사법의 적절한 시술방법과 기준의 설정에 대한 더 많은 연구가 필요하리라 사려된다.

Key Words : Fluorescence in situ hybridization, Chorionic villi sampling, Aneuploidy.