Fluorescence in situ hybidrization (FISH) A rapid method for sex determination in prenatal diagnosis

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Sex determination is of great interest in the prenatal diagnosis of X - linked inherited diseases. FISH technique allows the visualization of fluorescent labeled molecules at the sites of specific DNA suquences which were first hybridized with labeled probes. The technique can be applied to amniotic cells, lymphocytes, trophoblasts, sperm, solid tissues, hematological cancers and solid tumors. FISH allows direct visualization of DNA sequences of interest in metaphase or interphase nuclei. [Turk J Med Res 1993, 11(5): 228-230]

Key Words: FISH, Prenatal diagnosis, Sex determination

Sex determination is of great interest especially in prenatal diagnosis of recessive X- linked diseases. Recent progress in the techniques available for prenatal diagnosis have provided new opportunities for patients and their physicians to learn more about the fetuses and therefore to distinguish the abnormal ones from the normal. Patients are always very anxious to learn about their fetuses as early as possible in pregnancy. This has forced the investigators to find new methods which will be useful. The prenatal diagnosis of many disorders, such as Fragile X Syndrome, Cystic Fibrosis, hemoglobinopathies, in born errors of metabolism, numerical and structural chromosome aberrations is now possible with the use of molecular techniques one of which is fluorescence in situ hybridization (1-6).

With in situ hybridization techniques, specific nucleic acids can be detected in morphologically preserved chromosomes, cells and tissue sections (7,8). Sex determination can be carried out on intact fatal cells (free and cultured amniotic cells, chronic villus Lio.jsies, fetal blood cells) and adult cells (peripheral white blood cells, sperm, solid tissues).

Results can be obtained within 24 hours when freshly fixed cells are used. This procedure will surely accelerate the prenatal sex diagnosis and will be helpful to the physicians and the families for the final decision (1,4-8).

In this study FISH was used for sex determination in interphase nuclei and metaphase spreads obtained from amniotic cell cultures.

MATERIALS AND METHODS

Chromosome preparations: Amniocentesis was perfomed in 18th weeks of pregnancy transabdominal^. Cells from 10-20 ml of amniotic flued were cultured in plastic tissue culture flasks in HAM's F-10 supplemented with 20% fetal calf serum and antibiotics. After 12-14 days incubation the cells were treated with 0.05 ug/ml of colcemid for 2 hours and harvested by trypsination, fixed in ethanol: chloroform: glacial acetic acid=6:3:1 and slides were prepared. The slides were washed in PBS (3x3 min. each) to remove traces of acetic acid and put through ethanol series (70%, 80%, 90%, 100%, 5min. each). They were stored in 70% ethanol at 4°C until FISH was performed (2).

Direct preparation of amniotic cells in interphase: Cells taken directly from amniotic fluid were fixed in methanol/acetic acid (3:1) for 10 min, applied to glass slides and air dried (7).

DNA probe: The probe used in this study was biotin labeled human alpha satellite probe (DxZ1) specific for the X chromosome (ONCOR).

In Situ Hybridization: Slides were denatured in 70% formamide, 2xSSC (pH=7.0) at 70°C for 2 min. dehydrated in ethanol series (70%, 80%, 100%) at room temperature and air dried. The hybridization mixture (65% formamide, 10% dextran sulfate, 2xSSC)

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and the biotinylated probe was denatured at 70°C for 5 minutes in a water bath and applied to each slide. Hybridization was performed overnight at 37°C in a humidified chamber. Post hybridization washings were carried out at 43°C in 65% formamide, 2xSSC (pH-7.0) three times for 2 minutes each and then by 2xSSC at room temperature three times for 5 minutes each. The slides were then collected in 1xPBD.

Histochemical Detection: The slides were incubated with fluorescein-labeled avidin for detection with fluorescense. Propidium iodide was used as a counter stain together with antifade (p-phenylenediamin). The slides were finally covered with a glass coverslip and examined with a Nikon Microphot FX-A fluorescent microscope equipped with the specific filter combination (2,5).

RESULTS

In this article, we present our results on in situ hybridization of chromosome-X specific DNA probes to chromosome and interphase nuclei for sex determination in prenatal diagnosis in four cases. We applied the technique to both metaphase spreads (Figure 1) and interphase nuclei (Figure 2) obtained from amniotic cell cultures and to interphase nuclei in freshly fixed amniotic cells (Figure 3). In all experiments, we were able to demonstrate a single hybridization signal on the X chromosome showing the fetus is a male and the results obtained within 24 hours.

DISCUSSION

Fluorescent in situ hybridization (FISH) is a techinique which allows the detection of spesific nucleic acid sequences in cells or chromosomes. This process can

Figure 1. Metaphase spread from amniotic cell culture. Arrow indicates the X chromosome probed with biotin-labelled human a- satellite DNA probe (DXZI-ONCOR), counterstained with propidium iodide.

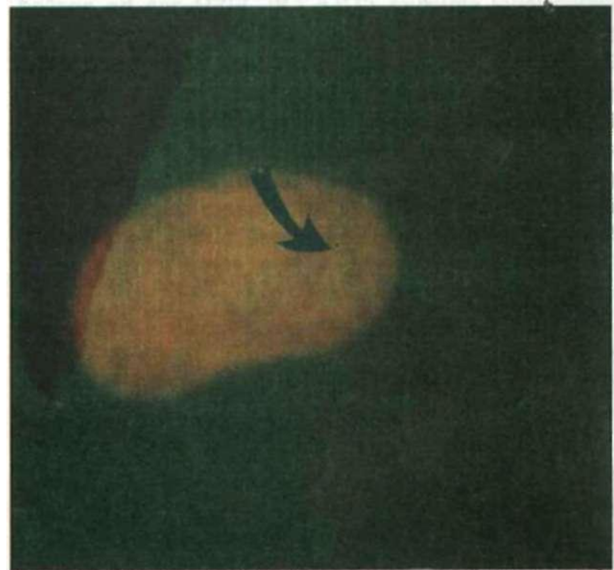

Figure 2. An interphase nucleus from cultured amniotic fluid cells. Arrow indicates the X chromosome probed with biotin-labelled human a-satellite DNA probe (DXZI-ONCOR) counterstain: Propidium iodide.

Figure 3. X chromosome in an interphase nucleus from directly fixed amniotic fluid (arrow). (Biotin-labelled human a-satellite DNA probe (DXZI-ONCOR) counterstain: Propidium-iodide).

be used to reveal the location of these sequences and to quantify their copy number. The technique can be applied to metaphase chromosome spreads and to interphase nuclei obtained from cells cultures, to freshly fixed interphase nuclei fixed on slides or to interphase nuclei fixed in suspension to preserve their three-

dimensional structure (2,3,5,7,8). FISH can be applied to different tissues such as amniotic cells, chronic villus biopsies, fetal or adult peripheral white blood cells, sperm, solid tissues, hematological cancers and solid tumors (2-14). In this study the technique is applied to chromosomes and interphase nuclei obtained from amniotic cells. In order to compare the results, in all cases, chromosomes and interphase nuclei obtanied from cell cultures and interphase nuclei from directly fixed cells were used and the same signals on X chromosomes were obtained.

FISH technique allows the deposition of fluorescent molecules at the sites of specific DNA sequences. DNA molecules are first labeled with reporter molecules such as biotin. In order to label them, target nuclei or chromosomes are denatured to produce single stranded DNA. The target is incubated with chemically modified single stranded nucleic acid sequence probes under conditions that promote reannealing of the probe with sequence in the target to which it is homologous. The probe is then detected using an avidin labeled fluorescent reagent that binds only to its chemical modifications (15,16,17).

The technique was originally developed about twenty pears ago by Pardeu and Gall (1969) (18) and independently by John et al (1969) (19). Since then, its application has expanded rapidly and now it is used successively in many different fields such as Cytogenetics, prenatal and tumor diagnosis, radiation studies and basic biology.

By using probes labeled with different fluorochromes, it is also possible to visualize signals in different colors for X and Y chromosomes in the same metaphase or in the same interphase nucleus (2,20).

Especially when directly fixed free cells are used, the results are obtained within 24 hours (2). In our experiments we also obtained our results in a day (20). We believe that this technique will facilitate and accelerate the prenatal diagnosis of many disorders including sex ones.

Floresan in situ hibridizasyon (FISH) prenatal sex tayininde hızlı bir metod

Cinsiyet tayini X kromozomu yoluyla geçiş gösteren hastalıkların prenatal tanısında büyük önem taşımaktadır. Yöntem amniyon hücreleri, lenfositler, trofoblast sperm, solid dokular, hematolojik kanserler ve solid tümörler gibi çeşitli dokularda, hem kültür sonucu elde edilen metafaz plaklarına, hem de taze materyalden elde edilen interfaz çekirdeklerine uygulanabilmektedir. DNA üzerindeki belirli bir nükleik asit dizisinin önceden işaretlenmiş bir prob ile hibridizasyonu ve bu bölgenin floresan bir boya ile görünür hale getirilmesi tekniğin esasını oluşturmaktadır.

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