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Identification of chromosomal-related abnormalities in Pfeiffer cell line using Fluorescence in situ hybridization

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Abstract

Studies that rely on fluorescence in situ hybridization (FISH) technique are of high quality and dependable results due to the fact that this technique is of high sensitivity, specificity, and speed which give FISH specific ability for detection of chromosomal abnormalities. FISH can be useful for detection of aneuploidy, characteristic gene fusions and loss of a chromosomal region. FISH can also be used as one of the beneficial tools in gene mapping and in detecting genetic abnormalities that might help in predicting cancers since they are considered to be precursors.

The aim of this work was to inspect the presence of chromosomal abnormalities involving chromosomes 14 and 18 in the Pfeiffer cell line using FISH technology with specified whole chromosome paint of the above chromosomes.

Key wards: FISH, bcl2 gene, chromosomal translocation

INTRODUCTION

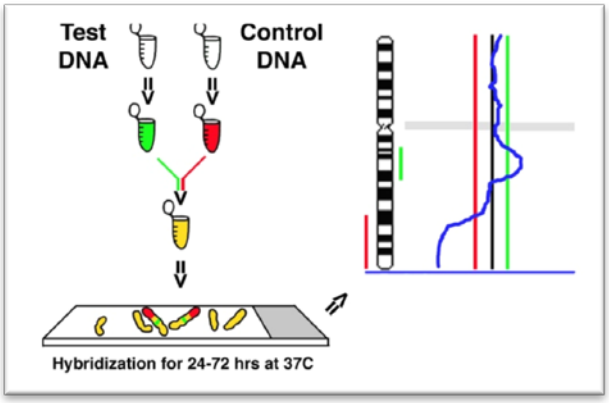
Fluorescence in situ hybridization (FISH) was first introduced in 1969. Since that time FISH has been used for determination of viral DNA sequence, mRNA and chromosomal regions. Nowadays, FISH is one of the most powerful techniques available for both biologist and pathologist and it is widely used as routine technique in many laboratories. (1)

FISH technology is based on the fact that single strand of DNA and RNA which labelled with fluorescence dye (probe) will hybridize to target molecular acids in neoplastic cells or tissues previously fixed on slides to form hybrids. In FISH techniques, the probe either labelled directly with the flourochrome such as Texas Red, FITC, Rhodamine, Cy2, Cy3, Cy5 and AMCA that are considered to be the most common. Or indirectly with a low molecular weight compound (hapten), and then subsequent amplification via a flourochrome conjugated antibody will be required including biotin that can be detected by streptavidin and digoxigenin that can be detected by specific antibodies. The direct technique is not as sensitive as the indirect one but it is quick and can be obtained with low background signal (1). In addition, the unique sequence probes, the whole chromosome paints, and the repetitive sequence probes are commonly used in FISH with specific applications for metaphase or interphase cells (1).

Although this technique has been widely used since more than 30 years ago, the design and use of FISH probes remain one of the highly desired techniques. New application were recently added to the procedure including the use of tyramides and the rolling circle amplifications to be more advantageous in detecting small DNA target sequences of less than 1-3kb.

Types of FISH technique includes fiber-FISH that is used to obtain high resolution mapping and determining the genomic rearrangements (2). Other types are known as Sky and M-FISH are useful in mapping of chromosomal breakpoints, unbalanced translocations awareness, marker chromosomes recognition in solid tumours and complex rearrangements characterization (3).

Comparative genome organization (CGO) is a molecular cytogenetic method for the detection of chromosomal imbalances (4), and the procedure is mentioned below as seen in schematic (1).



Schematic (1): Comparative genomic hybridization.

MATERIALS AND METHODS:

Pfeiffer cell line:

Those cells were cultured after being obtained from lymphoma cells that belonged to diffuse large cell lymphoma patients during the leukemic phase (5). This disease is characterized by the presence of several chromosomal abnormalities that have been studied in details previously.

Probes

A whole chromosome paint specific for chromosome 14 labelled with biotin was used. Since biotin has no colour, streptavidin conjugated with cy3 was added. In addition to whole chromosome paint that as specific for chromosome 18 was directly labelled with fluorescein isothiocyanate (FITC) which gives green colour. Then, the probe was denatured at 65C° for 10 min on a heat block and incubated at 37 C° for 10min.

Chromosome and nuclei preparations

Five ml of the cell culture was centrifuged at 1200 rpm for 5 minutes, and then the supernatant was discarded. The pellet then was re-suspended by vortexing. After that, 5ml of hypotonic solution (KCL 0.075) was added to obtain in wards movement of water across cell membrane. As a result the cells got swelled and chromosomes in the metaphase got separated for harvesting purpose.

The tube was incubated for 10 min at 37C° after that, 5 drops of fixation solution was added to block the action of the hypotonic solution. Again it was centrifuged at 1000rpm for 5 min the supernatant was removed, and the pellet was vortexed in 1 ml of the fixation solution to be re-suspended.

Five millilitres of fixation solution (75% of Methanol and 25% of Acetic acid) was slowly added. The tube then was put in incubator at room temperature for 15 min followed by centrifugation for 5 min at 1000 rpm. The resulted supernatant was got rid of in a glass bottle. The steps from resuspending to the discarding were repeated twice.

The slide was prepared by adding 5-8µl of the cell and chromosome suspention onto clean slide. Finally, nuclei quality and chromosomes spreads were examined by using microscope.

Denaturing of Chromosomal DNA

This procedure was started with using 2x SSC to wash the slide by shaking the Coplin jar for 5 minutes. Then, the slide was dried of through alcohol series as follow: 70% ethanol (ETOH), 90% ETOH, 100% ETOH followed by allowing the slide to dry for 5 min. The slide was incubated in denaturing solution in water bath (under fume hood) at 70C° for 5 min before it was put into 2x SSC Coplin jar followed by shaking in ice-cold 2x SSC for 5 min to be washed. After dehydration through alcohol series mentioned above, the solution was discarded following by allowing the slide to dry for 5 min.

Hybridization

To visualize the nucleic acids sequences in the cellular preparations to complementary probe sequences by hybridization, 10µl of probe that was denatured added to the already prepared slide and protected with a 22 x 22 mm coverslip. Then, the coverslip was insured to keep the slide in wet chamber overnight at 37 C°. Next day, the slide was passed through many washes: the first wash in 2x SSC for 5min and the purpose of this wash was to remove both the excess probe and the hybridization buffer from the slide. The second wash was in 0.4x SSC which assisted to eliminate the non-specific DNA hybridized to the chromosomes. After that, the slide was treated through many steps including incubation, washing, blocking, and finally treated with streptavidin and DAPI solutions before being stored sealed with nail polish.

Visualization of the signal

Few drops of DAPI were added to the slide to detect the labelled probe in the tissue by using fluorescence microscope.

RESULTS AND DISCUSSION

The results of this study shows that on the metaphase of the Pfeiffer line there was one chromosome partially painted while, the other three chromosomes were fully painted by using the WCP14 and WCP18.

The findings of the current study are consistent with those of Valia S., et al (2003)(6) who found that a small portion of chromosome 18 was translocated onto the distal region of long arm of chromosome 14. It is interesting to note that this practice has shown the occurrence of a der (14) chromosome partially painted with red colour because of the translocation of small segment from chromosome 18 onto distal region of q arm of chromosome 14 (represented by green colour). Also at the same time it is difficult to observe any translocation of genetic segment from chromosome 14 onto chromosome 18. Aschematic representation of the results obtained is shown in figure (2).

The absence of the translocation 14 onto 18 probably because of low hybridization efficiency or due to unbalanced translation that has happened and led to lose a part of the one arm of chromosome 18 as seen in figure (1).

The resolution of two fluorescence signals on a metaphase chromosome is about 1-2 Mb and this image which is obtained from this practical FISH is slightly beyond this resolution (1) as seen in figure 1(A and B).

In fact, the hybridization is affected by many important factors that have a fundamental role in the hybridization process such as temperature, duration of the hybridization time, the activity of the post-hybridization washes, pH, formamide, and salt concentration.

Generally, low temperature washes, high amount of probe, high salt concentration in the washing buffer and short hybridization time affect hybridization. All of these factors lead to low hybridization efficiency and increase in background that located mostly on chromosomes (7). In future, practical FISH might be possible to increase the hybridization time to compensate low binding efficiencies (8).

It can be clearly seen that the metaphase chromosomes have shown with blue colour due to the using of a counter stain (DAPI). Many red and green spots like starry have appeared in background located on chromosomes. A possible explanation for this phenomenon is that the probe fragments were too large or may be due to the contamination of the slide. In addition to that, the morphology of the chromosomes was poor and no clear banding. This result might be due to the occurance of overdenature that may have happened, but it may have something to do with the temperature and the time of denaturation (1).

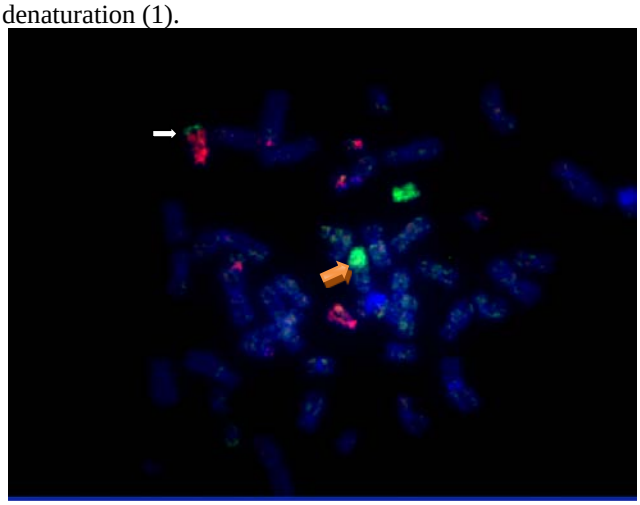


Figure 1: (A) Pfeiffer cell line metaphases hybridized with chromosome paints for chromosome 14 (red because of the red colour of Streptavidin conjugated with cy3) and 18 (green because of the colour of FTIC), DAPI is used as a counter stain to visualize the metaphase chromosomes, white arrow refers to the der (14).

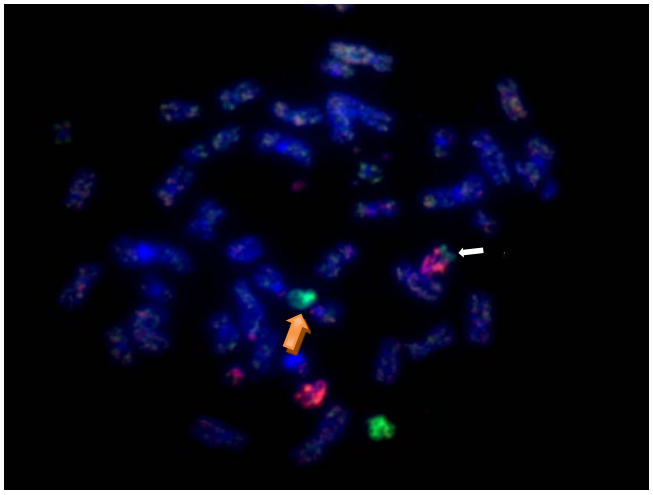


Figure 1 (B): White arrow refers to the unbalanced translocation of small portion of chromosome 18 onto chromosome 14, while orange arrow refers to the possible loss of the genetic segment from one arm of chromosome18.

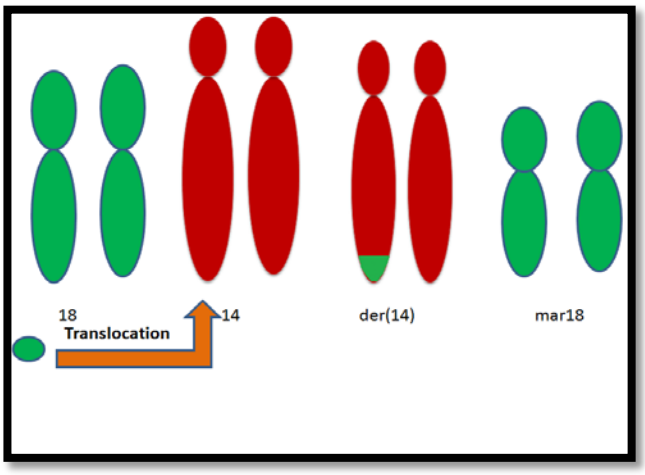


Figure (2): Schematic image of the chromosome 14 and 18 organized in the Pfeiffer metaphases using WCP14 and WCP18. The ideograms show two copies of each chromosome one copy of chromosome 14 only partially painted, which indicated as der(14) and this because it is possessing the centromere of chromosome 14, as the same time there is a significant genetic loss from one copy of chromosome 18.

The contiguity between bcl-2 gene on chromosome 18 and the immunoglobulin heavy chain (IgH) gene locus on chromosome 14 which resulted in result is bcl-2 overexpression might explain the translocation that was shown between chromosome 14 and 18 t (14; 18)(q32;q21) (9). In addition to that, myc proto-oncogen translocation has also reported in diffuse large cell lymphoma but it is not as significant as another chromosome abnormality (10). This translocation was found to be in about 85% of primary nodal follicular lymphomas and 30% of high-grade diffuse large B-cell lymphomas and involved in their pathogeneses (9). Moreover, this translocation was also detected in B cells of patients with non-Hodgkin's lymphoma (11).

The present findings seem to be consistent with other research which found that structural abnormalities in chromosome 14 were more frequent than numerical alterations and the most common are t(14;18)(q32;q21) in diffuse large cell lymphoma (12). These findings further support the other result of another research which have illustrated that this translocation is a stable result in follicular lymphoma (FL) (13).

Being considered as the primary tumorigenic event in FL and diffuse large B-cell lymphomas (DLBCL), this translocation is important in most FL and some DLBCL (14). This importance comes also from the fact that it is considered to be indicator of lymphoma depending on the number of positive cells in peripheral blood that when increases during remission predict expectant relapse (15). Those researchers utilized this translocation to recognize positive cells before and after bone marrow transplantation.

Furthermore, there is another translocation between chromosome 11 and chromosome 18 has been detected in DLBCL by using in situ hybridization on formalin-fixed, paraffin-embedded specimens (16). On the other hand, the findings of the practical report do not support the results of a previous research which have established that there was absence of a t(14;18) in many patients with high-grade cutaneous DLBCL although some of them they were Bcl-2 positive (17).

In conclusion, using FISH technique can be very helpful in detection of translocation t(14;18)(q32;q21) in the Pfeiffer cell line that plays significant role in the diagnosis of DLBCL and can be used to examine therapy response and level of remaining disease. Further researches should be done to investigate other chromosomes that are involved in the translocation in different types of malignant lymphoma.

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