Medical Applications

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Classical & Molecular Cytogenetics as a Tool for Clinical Investigation of Genetic/Acquired Abnormalities

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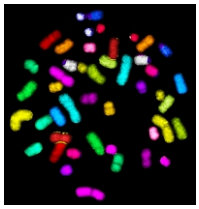

Metaphase spread probed with 24 color multiplex-FISH

ABSTRACT

In this study, a comparative evaluation of classical and molecular cytogenetics is made with special emphasis on advancement of Fluorescence in-situ hybridization (FISH) based techniques. Cytogenetic investigation of a rare genetic abnormality, wiskott aldrich syndrome (WAS), was carried out in a male child. Results indicated that the subject was prone to genetic instability when challenged with radiation stress. Another case of male-sterility was cytogenetically analyzed, wherein the abnormality detected was the presence of two spots corresponding to sequences of Y-chromosome and this may contribute to the onset of infertility in the individual.

KEYWORDS: *Molecular cytogenetics, Fluorescence in-situ hybridization (FISH), Multiplex-FISH, Clinical cytogenetic-investigations, Wiskott aldrich syndrome, Male-sterility*

Introduction

Cytogenetics deals with the chromosomal studies, where in fine structure, number and behavior of chromosomes are analysed and related with the respective functions. It defines how structural and numerical chromosomal-abnormalities/changes, lead to development of genetic diseases/abnormalities (inherited and acquired) [1]. Cytogenetics is not a new branch of science, it is deeply rooted in genetics and cytology [2]. With the advancement of precise controlling methods on cell cycle-regulation, preparation of chromosomes, removal of non-target interfering molecules from the cell, development of highly precised DNA/RNA binding fluorescence probes and refined fluorescence techniques for fine capturing and analysis of structural details, cytogenetics has gained a significant advancement in current status of science [3]. Cytogenetics has gained substantial attention in recent years in the field of clinical investigations, for example, understanding the molecular basis of inherited and/or acquired genetic abnormalities, emergence and development of malignancies and cell transformations induced by any physical, chemical or biological agents [4].

Classical Cytogenetics

Conventional cytogenetics had its own glorious era, it was extensively-explored for diagnosis and prognosis of wide spectrum of inherited and acquired genetic abnormalities for several decades [5]. It has also been extensively used for characterization of specific chromosomal aberrations and their correlation on development of clinical syndromes for instance mental retardation, developmental delay, congenital and other anomalies [7]. Conventional karyotyping methods, considered as gold standard, depends on; 1) Giemsastaining of whole chromosome (continuous staining, Fig.1A) and 2) Banding of chromosomes (G and C banding using Giemsa, Fig.1B). These methods have potential to quantify loss or gain of comparatively bigger (in mega-base pair range) portion of genome, their rearrangements within and between the chromosomes [6]. Metaphase cytogenetics is a major contributor to understand the genetic basis of cancer, its monitoring and tumor staging [8]. In addition, classical cytogenetics has significantly contributed in the field of biodosimetry of planned and unplanned radiation exposures such as medical and accidental exposures [9].

However, conventional cytogenetics is a time consuming and labor-intensive process. It majorly relies on Giemsa staining, which is limited by poor resolution (Fig.1). Clinically relevant submicroscopic chromosomal abnormalities (in kilobase pair range) such as, minorloss (deletion) or gain (insertion) or rearrangements (within or between the chromosomes) remains undetectable [10]. Primarily, a trained or expert cytogeneticist is required to analyze and karyotype G-banded chromosomes [11].

Molecular Cytogenetics

Molecular cytogenetic techniques have evolved as an indispensable addition or even improved alternative to conventional cytogenetics; it enhances interpretation of numerical and complex structural chromosomal aberrations by bridging the gap between conventional and molecular cytogenetics (12).

FISH based molecular cytogenetic techniques can precisely detect, qualitatively and quantitatively, microscopic and submicroscopic genomic changes (in kilobase range, i.e., ~50-100-fold higher than conventional Giemsa staining). With the advanced FISH based techniques, such as development of locus specific and whole chromosome painting probes, spectral karyotyping (multiplex-FISH and multi-BAND-FISH) and Comparative Genome Hybridization (CGH), molecular cytogenetics have emerged as highly-effective diagnostic and research tool (13).

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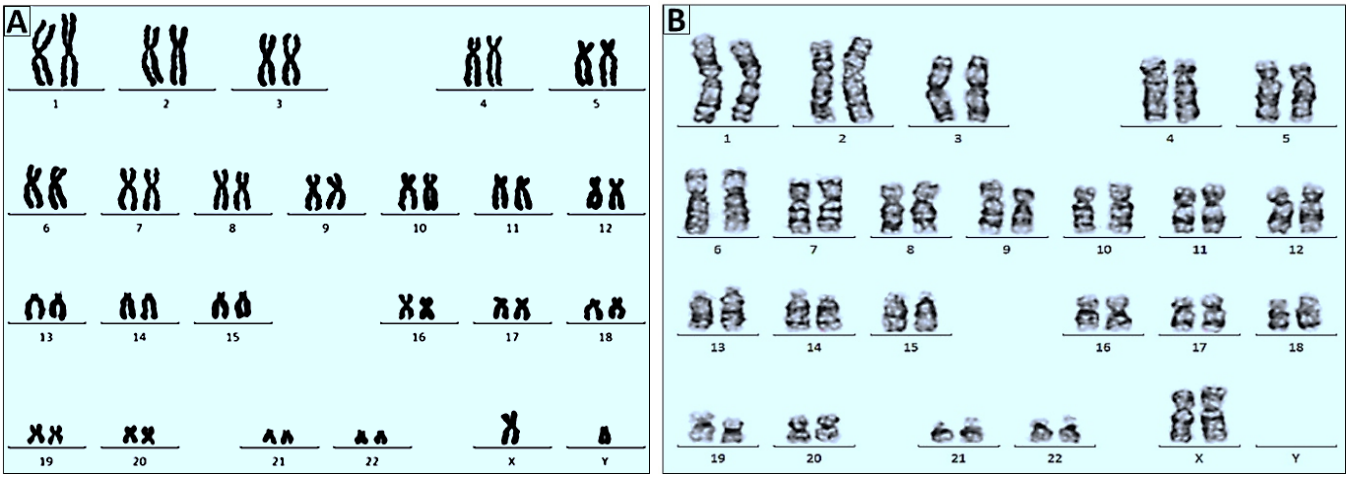

Fig.1: Representative illustrations of (A) A control karyotyped metaphase, having no aberration, stained with Giemsa and (B) A control karyotyped G-banded metaphase, having no aberration, stained with Giemsa.

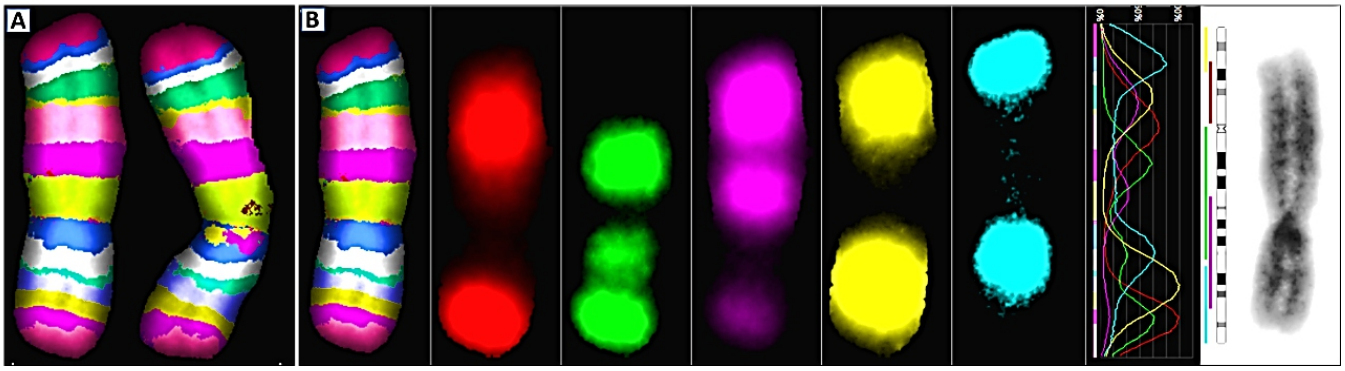

Fig.2: Representative illustrations of (A) A metaphase chromosome pair (second pair), probed with multi-BAND-FISH probes, different regions of the same chromosomes are painted with probes of different colors and (B) Mono-color karyo-gram of chromosome (second pair), observed under different set of filters (total five set of filters were used).

Until recently, detection, identification and quantification of intra-chromosomal changes was limited by poor resolution, as it was based on classical G or R-banding technique. G and R banding is limited to just two alternate light and dark banding patterns. It is tedious and perplexing to identify and quantify, intra-chromosomal and/or complex changes/ rearrangements, involving different regions of the same chromosome or different regions of the different chromosomes. An advanced FISH based technique; mBAND-FISH has made this job easy by creating a series of colored bands along the axis of the subject chromosome, which is easy to identify and quantify (Fig.2). It is based on applying region specific partial paint probes, linked with quantitative color ratio analysis (carried out by automated software, ISIS) [14, 15]. Another advance tool, multiplex FISH, covers whole genome, paints all 24 types of human chromosomes with different colors and facilitates detection and quantification of microscopic changes that are genome-wide (Fig.3). FISH based techniques has widened its horizon to multiple directions aiming at different applications by targeting sequences of DNA and RNA [16].

Clinical investigations of human genetic abnormalities

Cytogenetic techniques have been established at the Biodosimetry Laboratory of RPAD, BARC, primarily for biodosimetry of excess exposures of radiation workers to low and high LET radiation. Nonetheless, these techniques are equally useful in evaluation of various clinical parameters. To demonstrate the potential applications of these techniques in clinical investigations, the established cytogenetic techniques and their dose response curves (generated for various biological indicators) were employed for two cases of inherited genetic abnormalities/instabilities namely, (1) An immunodeficient, wiskott aldrich syndrome (WAS) patient, and (2) A genetically sterile male.

Case study-I: Cytogenetic analysis of wiskott aldrich syndrome:

Wiskott Aldrich Syndrome (WAS) is an X-linked primary immunodeficiency disorder. The gene responsible for WAS is located on the short arm of the X chromosome at Xp11.22–p11.23 [17]. Abnormalities in cell-mediated, humoral, and innate immunity have been observed in WAS patients. This abnormality in immune system majorly involves T lymphocytes. WAS patients are associated with both quantitative and qualitative defects in T and B cells [18,19]. Serum levels of immunoglobulin IgG, IgM, and IgA are often low and IgE levels are often high in patients with WAS [20]. Mutation in WAS gene affects the synthesis and/or activity of WAS protein, this may lead to immunodeficiency, X-linked-neutropenia, autoimmunity, X-linked thrombocytopenia, genomic instability and sometimes hematologic malignancy too [21]. In this study, genetic instability in the blood sample of a immune-deficient male patient suffering from WA-syndrome was carried out by cytogenetic end points, employing reciprocal and nonreciprocal translocations, dicentrics and chromosomal fragments.

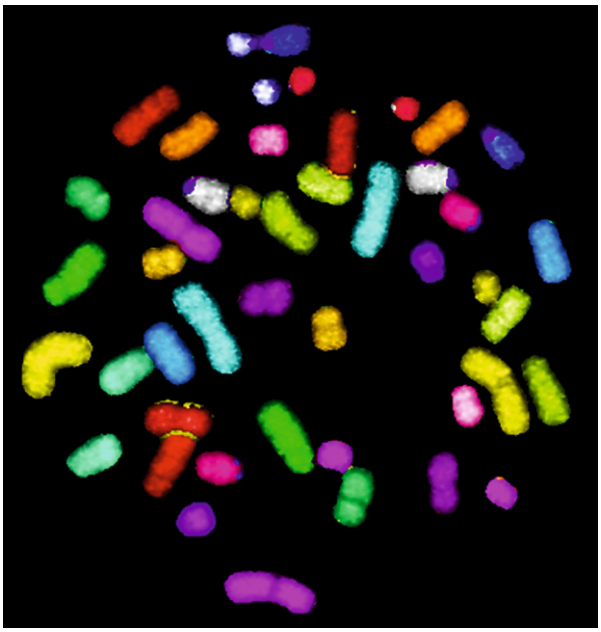

Fig.3: Representative illustration of a metaphase spread probed with 24 color multiplex-FISH; all pairs of the chromosomes are painted with probes of different colors.

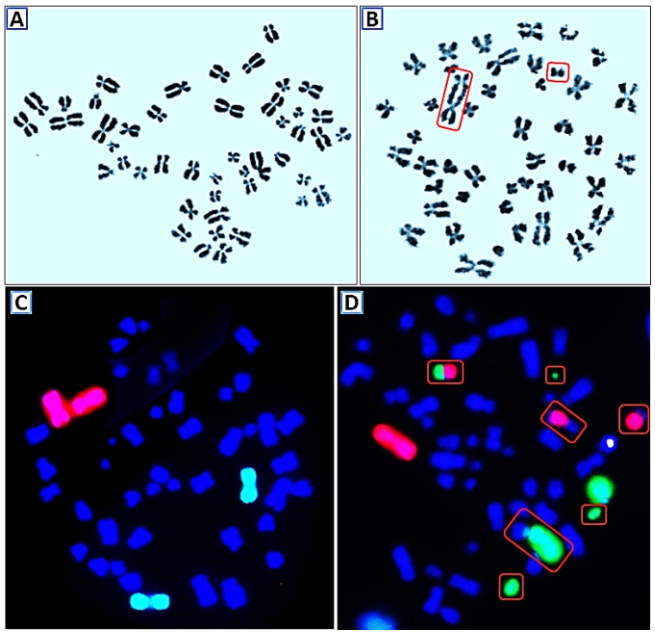

Fig.4: Representative images of lymphocyte-metaphase spread obtained from wiskott aldrich syndrome patient (A) Giemsa stained, control metaphase with no radiation exposure, (B) Giemsa stained metaphase (exposed with 2 Gy) with a dicentric and a fragment. Metaphase spread processed with two color FISH for chromosome pair one and two (first pair hybridized with green probes and second pair hybridized with red probes) (C) Control metaphase with no radiation exposure and (D) Metaphase (exposed with 2 Gy) with multiple aberrations, viz., reciprocal and non-reciprocal translocations, chromosomal fragments involving painted chromosomes.

Observations

Spontaneous and radiation induced chromosomal aberrations were analyzed in the lymphocytes of the WAS patient and a comparison was made with the lymphocytes of a healthy volunteer (control). No difference in the background frequency of dicentrics and chromosomal fragments was observed. Instead, the unirradiated WAS cells possess considerably high yield of chromatid breaks, (~ 5%) in comparison to control sample (none in 500 metaphases analyzed). The reason for presence of excess chromatid break is not very clear, inherent genomic instability in WAS (22), could be the probable reason. Irradiated (2 Gy) WAS sample showed 51% excess yield of dicentrics (Fig.3A and B), 59.1% excess yield of reciprocal translocation (Fig.3C and D) and 80% excess yield of non-reciprocal translocation, in comparison to blood-sample obtained from healthy individual irradiated with the same dose. Dicentrics and translocations are formed as a result of mis-repair of DNA double strand breaks. Overall, 51 to 80% excess mis-repair events were observed in WAS lymphocytes over control lymphocytes. These results indicate that lymphocytes of WAS patients are more sensitive towards radiation in comparison to control lymphocytes and are less efficient in maintaining their chromosomal integrity. These finding are in agreement with recent reports, deciphering R-loop mediated genomic instability in WAS patients [23]. In addition, γH2AX foci decay kinetics has also shown that, foci persistence is significantly higher in number up to 48 h, after irradiation (data not shown here). In the view of above outcomes, it can be concluded that high radio-sensitivity/ genetic instability can lead to higher level of cellular transformation or lethal aberrations and/or other genetic complications in WAS patients.

Case study-II: Cytogenetic investigation of a genetically sterile male:

Sterility in males is not a very rare phenomenon, 7% of the male population suffers with this problem [24]. The aetiology of infertility is majorly known in two-third of the affected population while, in one-third population it still remains unidentified. The usual reason for sterility in males is, chromosomal aberrations (numerical or structural), infection and/or abnormal gland functioning and over exposure of some environmental stresses [25]. 2 - 14% sterile male population possess chromosomal abnormalities (structural and/or numerical) [26] indicating that chromosomal abnormalities may have profound role in progression of sterility in males.

In this study, cytogenetic analysis, to check the integrity of the chromosomes, was carried out in the blood sample of a subject suffering from infertility (unable to reproduce). Subject was in his fertile age. Intactness/integrity of chromosomes were analyzed by G0-FISH and two color-FISH.

Observations

Cytogenetic analysis, to evaluate the integrity of the chromosomes was carried out in the blood sample of a male subject, using FISH and a comparison was made with the blood sample of a healthy male volunteer. G0-FISH for chromosome-Y was carried out in isolated and unstimulated lymphocytes. Results have shown that subject lymphocytes were possessing two red spots corresponding to Y-chromosome (one large and one small, red spot), per cell (Fig.5B), though control lymphocytes possess only one large red spot per cell (Fig.5A). These findings indicate the presence of two Y-chromosomes of different sizes in each lymphocyte of the subject, which is not normal.

Further analysis was carried out with the metaphase FISH, using whole chromosome paint probe for chromosome-Y in the blood sample of the subject and the healthy control volunteer. Again, two red spots (one large and one small)corresponding to Y-chromosome was observed in metaphases too. Upon analysis of Y-chromosome in metaphases, it was observed that morphology and integrity of Y-chromosome was slightly altered, p arm appeared to be a little shorter than the usual size, as indicated by large red spot observed in G0-FISH. It was also observed that a small region

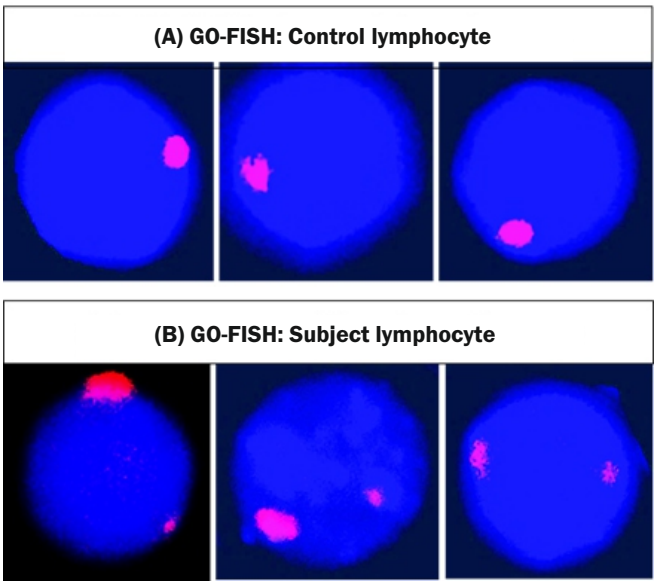

Fig.5: Interphase lymphocytes with G0-FISH, painted with whole chromosome paint probe for Y-chromosome. (A) Lymphocytes from a healthy volunteer (control) with one spot per cell for Y-chromosome. (B) Subject lymphocytes (sample) with two spots of Y-chromosome (one large and one small, red spot) per cell.

of Y-chromosome was translocated to C-group chromosome. This observation, supports the small red spot observed in G0-FISH. As observed, Y-chromosome is morphologically altered and the additional small spot observed may be attributed to translocation of a small region of Y-chromosome with C-group chromosome. In case of control sample (healthy volunteer), only one large red spot, with intact morphology of Y-chromosome, was observed, as in G0-FISH, which is normal. No translocations or morphological changes are observed in metaphase of normal individual.

Terminal region of p-arm of Y-chromosome, possess crucial sex determining genes (SRY genes) and hence is pronounced as sex-determining region of Y (27). In this case study, sex-determining region of Y is translocated to C-group chromosome, this change in the location of these crucial genes, may be affecting the functioning of these sex determining genes and may be contributing to genetic basis of sterility in the subject. Further investigations may be warranted to make final conclusion of this case study.

Conclusion

In former decades, innovative technical advancements in cytogenetics, have substantially enhanced the detection and quantification of chromosomal aberrations/changes and potentially-facilitated in clinical and non-clinical investigations. FISH based techniques, in combination with karyotyping, have played central role in these technical advancements. Molecular cytogenetics have expanded beyond FISH, such as, comparative genomic hybridization (CGH), which offers genome wide screening with better resolution and high precision.

In this study, cytogenetic investigation clearly demonstrated genetic instability in a pediatric case of WA-syndrome and chromosomal basis of sterility in a male patient. Similarly, other human genetic abnormalities like, Severe Combined Immunodeficiency (SCID), Fanconi anaemia and different forms of malignancies can be investigated employing these advanced cytogenetic techniques to understand the genetic/chromosomal basis and possible consequences of these sever diseases/syndromes.

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