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Comparative Analysis of the Effects of Different Chromosome Preparation Methods on Nuclear Morphology and Chromosome Structure

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Abstract: Preparation of high quality chromosome samples is critical in cytogenetic research for accurate karyotyping and understanding cell biology. This paper examines the effects of different chromosome preparation methods on nuclear morphology and chromosome structure. It reviews the importance of chromosomes in genetics and cell biology and emphasizes the importance of high quality samples in karyotyping. Factors affecting chromosome quality include culture medium, processing intensity, chemical composition of fixatives, preparation methods, temperature, humidity, and airflow control. Evaluation of chromosome quality includes mitotic index, abnormal karyotype detection rate, and preparation success rate. Fluorescence in situ hybridization (FISH) is emerging as a promising high-resolution cytogenetic detection method, particularly for the detection of chromosomal abnormalities. This study examines the effects of different preparation methods on nuclear morphology and chromosome structure, providing insights into cell biology.

Keywords: Chromosome Preparation Methods; Chromosome Structure; Microscopy Techniques; Meiotic Chromosomes; Biological Sample Preparation.

1. Introduction

Chromosomes serve as carriers of genetic material within eukaryotes during cell division. Chromosomes have long been the focus of research in genetics and cell biology, and have also given rise to the development of the professional field of cytogenetics. Preparing chromosome samples is one of the fundamental techniques in cytogenetic research. In karyotyping, obtaining high-quality chromosome samples is crucial for accurate karyotyping. An ideal chromosome sample should have even dispersion, non-overlapping, clear resolution and banded texture. However, the quality of chromosome samples depends on many factors, including the quality of the culture medium, the intensity and time of the processing process, the proportion of chemical substances in the fixative, the preparation method, the temperature and humidity of the preparation environment, and the control of air flow. Commonly used indicators for assessing chromosome quality include mitotic index, abnormal karyotype detection rate, and chromosome preparation success rate. Normally, there should be at least five analyzable cell division figures on one slide, and the proportion of analyzable division figures should be high for chromosome preparation to be considered successful. The evaluation of indicators such as mitotic index and abnormal karyotype detection rate is based on the quantity and quality of analyzable mitotic figures. In addition to the above evaluation indicators, other factors need to be considered, such as the health of the cells, the stability of the culture conditions, the choice of chromosome staining, and the technical level of the experimenter. The health of cells directly affects the morphology and structure of chromosomes, so special attention should be paid to cell culture and handling during chromosome preparation. Maintaining the stability of culture conditions helps maintain the consistency of various parameters, thereby improving the success rate and accuracy of chromosome preparation. The choice of chromosome staining method depends on the purpose and requirements of the study, because different staining methods may have different effects on the structure and morphology of the chromosomes. Finally, the technical level of the experimenter also has a direct impact on the accuracy of the experimental results, so good experimental skills and experience are required.

Preparation of high quality chromosomal samples is critical for cytogenetic research to ensure accuracy and reliability of karyotyping. In practice, the various factors need to be taken into consideration and appropriate measures need to be taken to improve the quality and success rate of chromosome samples. In the Figure 1, several chromosome images are demonstrated.

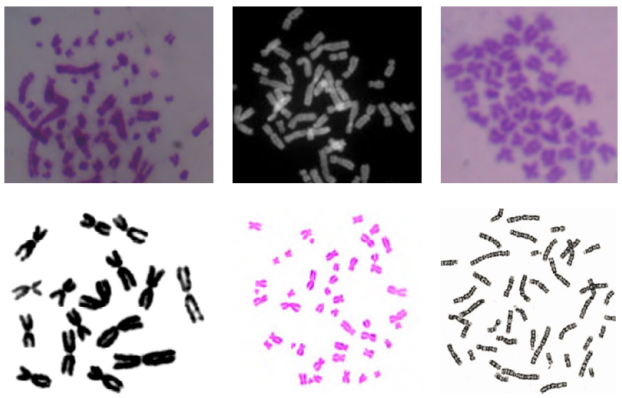

Figure 1. The Sample Chromosome Images

In the following sections of the manuscript, we will comprehensively examine how different chromosome preparation techniques affect both nuclear morphology and chromosome structure. Through detailed analysis, we aim to

elucidate the influence of different methods on these crucial aspects of cellular biology.

2. Literature Review

The literature review segment of this manuscript provides a comprehensive exploration of nuclear shape, chromosome movements, and nuclear arrangement, offering insights into core cellular processes and the origins of diseases. Guidi et al. [1] initiated a groundbreaking inquiry into the effectiveness of a cellulose-based nanomaterial for purifying freshwaters contaminated by heavy metals, particularly focusing on the effects of cadmium-induced DNA damage, shifts in cell growth, and changes in chromosome structures. In tandem, Fischer [2] illuminated the importance of nuclear appearance in identifying cancerous cells, delving into the molecular complexities that underlie unusual morphological features, which have served as pivotal diagnostic tools for cancers over a lengthy span. Strom et al. [3] provided invaluable insights into the role of HP1 as a connector of chromatin, elucidating its regulatory control over the mechanics of nuclei and chromosomes during cell division, thus advancing our comprehension of chromatin organization and its functional implications.

Moreover, Patil and Sengupta [4] presented an extensive review on the proteins comprising nuclear lamina, outlining their essential roles in nuclear structure and explaining the nuanced functions of A- and B-type lamins in modulating various nuclear processes crucial for cell function and stability. Meanwhile, Smith et al. [5] shed light on the crucial role of RNA molecules in organizing the nucleus and controlling gene activity, offering fresh theories on genome structure and the dynamic nature of nuclear organization. Schonhoft et al. [6] introduced an innovative marker for measuring chromosome instability in tumor cells circulating in the blood, providing critical insights into cancer progression and treatment outcomes, particularly in cases of prostate cancer.

In addition to these findings, Mirny and Dekker [7] explored the complex mechanisms involved in the folding of chromosomes and the organization of the nucleus, uncovering the intricate interplay between the dynamics of the chromatin and the architecture of the nucleus. Belmont [8] provided a broad overview of the compartments within the nucleus and the arrangement of genetic material, highlighting recent technological advancements in imaging methods and suggesting promising avenues for the future research. Smith et al. [9] expanded our understanding of the mechanisms that confer resistance to paclitaxel, focusing on the role of the nuclear envelope in mediating drug resistance in cancer cells, thus laying the groundwork for innovative therapeutic approaches.

Furthermore, Bera and Sengupta [10] investigated the relationship between nuclear structures and the positioning of chromosomes, offering insights into the regulatory mechanisms governing gene activity and genome stability. Janssen et al. [11] provided a comprehensive review of methods for quantifying abnormalities in nuclear shape, emphasizing the importance of precise measurement in diagnosing diseases and conducting research. Finally, Warecki et al. [12] elucidated the role of a specific protein complex in facilitating the movement of chromosomes through channels in the nuclear envelope during cell division, revealing novel mechanisms underlying nuclear stability and genome integrity, thus advancing our understanding of the fundamental cellular processes. Taken together, these studies represent significant contributions to our knowledge of nuclear biology and provide valuable insights into cellular processes and also disease pathogenesis. The multifaceted exploration of nuclear shape, chromosome dynamics, and nuclear organization presented in these studies underscores the complexity of cellular processes and points to potential avenues for future research and therapeutic intervention.

3. The Proposed Model

3.1. The Overview of Nucleus Morphology and Chromosome Structure with Sample Discussions

Fluorescence in situ hybridization (FISH) is an emerging cytogenetic detection method. Compared with traditional cytological detection methods, FISH technology has the advantages of rapidness, accuracy, and high sensitivity. Today, it has been widely used in the field of prenatal diagnosis, especially in the rapid detection of common chromosome number abnormalities, such as non-invasive prenatal genetic testing (NIPT) [13]-[15]. As a diagnostic-like screening tool, NIPT has been widely used in clinical practice and is favored for its high sensitivity and specificity. However, there is a certain degree of false positives and false negatives in NIPT test results. Among them, false positive results may result from abnormalities in maternal cell-free DNA, while fetal cell-free DNA is normal. For example, pregnant women may have somatic mosaicism, copy number variations, etc., or have received blood transfusions, transplants, cell therapy, or have tumors. These factors may lead to false positive results. Fetal cell-free DNA mainly comes from placental trophoblast cells, but in about 1% of cases, the chromosomes of placental trophoblast cells may be inconsistent with the fetal chromosomes. Even if the fetal chromosomes are normal, the trophoblast cells may be abnormal, resulting in false Generation of positive results. This discordance may be caused by restrictive placental mosaicism and disappearance of one of the twins. About 10% to 15% of the free DNA in maternal blood circulation comes from the fetus. Its level increases with the growth of the pregnancy cycle and is completely degraded 2 hours after delivery. If the gestational age at the time of NIPT testing is small, fetal cell-free DNA levels are low, and maternal cell-free DNA may mask fetal DNA, resulting in false negative results. In addition, the interpretation of results is also affected by the threshold setting, and misjudgments may occur. Therefore, understanding these potential factors is critical to correctly interpreting NIPT results.

Compared with traditional chromosome banding technology, FISH technology has a higher resolution, so it has become one of the key technologies in the detection of chromosomal abnormalities. In many cases, chromosomal abnormalities in translocation carriers tend to be complex, with one derivative chromosome likely to be clear while the other is difficult to detect. Even in metaphases with good G-banding quality, small differences between normal chromosome 7 and derivative chromosome 7 can be easily missed due to the relatively long chromosome 7. However, using fluorescence in situ hybridization with a chromosome 7-specific probe, we clearly observed that a portion of chromosome 7 was translocated to the end of the long arm of chromosome 18. Combined with the banding chromosome results, we can clearly see that this case is a carrier of a cryptic

translocation involving chromosomes 7 and 18. In another case, although G-banding identified her as a carrier of the reciprocal translocation, the breakpoint at the end of the long arm of the chromosome 7 made it difficult to determine. Therefore, in cytogenetics, it is particularly important to detect abnormalities in the terminal regions of the chromosomes. Abnormalities in the terminal regions of chromosomes directly affect chromosome stability, and reciprocal translocations often involve the terminal regions of chromosomes. The study found that tiny translocations in the terminal regions of the chromosomes are more common than previously thought. Cryptic subtelomeric monomers can explain 5% to 8% of unexplained mental retardation. By testing two cases, this article confirmed that one was a cryptic translocation in the terminal region, and the break-point of the other translocation did not include the subtelomeric region. This provides new cases for the study of chromosome terminal structural abnormalities and enriches our understanding of chromosomal abnormalities. The Figure 2 shows the sample results.

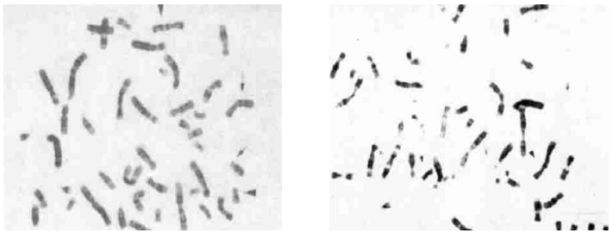

Figure 2. The Sample Results for G-banded karyotype of cases 1 and 2

3.2. The Comparative Analysis of the Effects of Different Chromosome Preparation Methods

For the comparison analysis, the preparation of lower animal chromosomes by "resuspension method" is selected. The detailed steps are:

(1) Cultivation of regenerated tissue: Gently cut off healthy insect bodies that have been starved for about a week before and after the pharynx, and use a single-sided blade to complete this process (see Figure 1 for details). Place the obtained fragments in a petri dish, add chlorine-free tap water, keep the temperature at about 15°C (indoor temperature in winter is sufficient), and cultivate for 3 to 5 days.

(2) Isolate the regenerated tissue: Use a brush to gently transfer the well-regenerated fragments to the Petri dish cover, then use a single-sided blade to cut out the regenerated fragments of about 1 mm, and place them in a clean Petri dish containing chlorine-free water. middle. The remaining insect body fragments can be cultured for other experiments on regeneration.

(3) Treatment with colchicine: Put the isolated regenerated tissue into a small amount of 0.02% colchicine solution (should be able to cover the material), and then place it in an incubator at about 15°C for 1.5 to 2.5 hours. . Observe the state of the material and give priority to a tissue that is approximately round or oval and has no disintegration.

(4) Implement hypotonic treatment: rinse the well-treated regenerated tissue with 0.1% potassium chloride (KCL) solution 2-3 times, then add a small amount of 0.1% KCL solution, and process it in an incubator at about 15°C About 1 to 1.5 hours.

(5) Centrifuge and resuspend: Transfer the hypotonic treated tissue to a 2ml centrifuge tube and crush gently with a sterile pin. Then add 1 ml of Type I fixative, shake for 30 seconds, and then centrifuge at 1000r to remove the supernatant; then add Type II fixative and repeat the above steps once.

(6) Separate cells after fixation: Add 1 ml of Type III fixative solution to the above-mentioned centrifuge tube, shake for 30 seconds, drop the mixture onto a clean glass slide, and then dry at room temperature.

(7) Dyeing: Dye with 5% Giemsa dye for about 10-15 minutes, then gently wash away the excess dye with distilled water, and dry at room temperature.

(8) Encapsulating slides: Preliminarily observe the smear with well-dispersed chromosomes under a microscope, and then use an appropriate amount of diluted neutral gum (xylene) to encapsulate it for storage.

In the considered method, the detailed information is:

This study collected 1,000 peripheral venous blood specimens and used a variety of experimental equipment and consumables, including the CDS-5 dispersion instrument from Thermotron and the Axio Imager Zeiss & Meta systems from German Metas Software and Hardware Technology Co., Ltd. Automatic scanning chromosome image analysis system, CO2 incubator from Thermo Fisher Scientific (China) Co., Ltd., wash-free slides provided by Shanghai Lechen Biotechnology Co., Ltd., lymphocyte culture medium from Guangzhou Dahui Biotechnology Co., Ltd. , colchicine and Giemsa stains, potassium chloride from Guangxi Xilong Scientific Co., Ltd., methanol and glacial acetic acid from Sichuan Xilong Scientific Co., Ltd., and trypsin from GIBCO. The experimental method includes the preparation of cell suspension, collecting samples from peripheral venous blood and inoculating them into peripheral blood lymphocyte culture medium, culturing in an incubator at 37°C for 72 hours, and then adding colchicine to continue culturing for 0.5 hours. The harvested cells were treated with 0.075 mol/L potassium chloride hypotonic solution, pre-fixed once with methanol and glacial acetic acid, and formally fixed twice. Finally, the cell suspension was made and placed in a refrigerator at 4°C overnight. After fixing again the next day, prepare a cell suspension of a certain concentration based on the amount of cell sedimentation for preparation of droplets. During the preparation process of drop tablets, Thermotron's CDS-5 dispersion instrument was used to prepare drop tablets under different temperature and humidity conditions. In the staining and observation stage, the slides were subjected to digestion banding and Giemsa staining, and were scanned using the Axio Imager Zeiss & Meta systems fully automatic scanning chromosome image analysis system. The analysis results involve the success rate of chromosome preparation and the number of cleavages with less than 3 chromosome overlaps and the percentage of cleavages available for analysis. Repeat the study on 10 cell suspension samples, and average the final results to determine the best combination of temperature and humidity. Success rate of chromosome preparation under different temperature/humidity combinations is selected as the factor for evaluating the performance. In the Table 1, the experimental results are shown.

Experimental results show that under different relative humidity conditions, there are certain changes in the success rate of chromosome preparation. When the relative humidity is 40%, the success rate of chromosome preparation is 55.7%; when the relative humidity increases to 45%, the success rate

of chromosome preparation increases slightly, reaching 57.9%; with further increase in relative humidity, the success rate of chromosome preparation It also gradually increased, with a success rate of 60.2% under 50% relative humidity conditions. However, when the relative humidity reached 55%, the success rate of chromosome preparation reached 63.1%, and then at 60% and 65% relative humidity conditions, the success rate slightly decreased to 62.9% and 61.4%, respectively. Comprehensive analysis shows that the success rate of chromosome preparation has a certain positive correlation with relative humidity, that is, as the relative humidity increases, the success rate of chromosome preparation also increases accordingly. However, within a certain range, too high or too low relative humidity may have certain negative effects on chromosome preparation. Therefore, appropriate relative humidity conditions are one of the key factors to ensure successful chromosome preparation.

Table 1. The Experimental Result

Relative humidity (%)	Chromosome preparation to success (%)
40	55.7
45	57.9
50	60.2
55	63.1
60	62.9
65	61.4

4. Conclusion and Future Scopes

This study elucidates the influence of different chromosome preparation methods on nuclear morphology and chromosome structure. Comparative analysis reveals that relative humidity significantly affects the success rate of chromosome preparation, with higher humidity correlating with increased success rates up to a certain threshold. Beyond this threshold, excessively high or low humidity may adversely affect chromosome preparation. Future research should explore optimal humidity conditions and investigate additional factors that affect chromosome quality. In addition, the study highlights the importance of high-quality chromosome samples in cytogenetic research and clinical diagnostics. Further research can explore advanced techniques to improve chromosome preparation methods and increase the accuracy of karyotyping. In addition, exploring the implications of chromosomal abnormalities detected by FISH technology opens avenues for understanding genetic disorders and advancing personalized medicine.

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