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The Spatial Location of Chromosomes in Dividing Cells and the Relative Stability of Chromosome Spatial Structure

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Abstract: The stability and evolution of human genetics depend on chromosomes and chromosome-chromosome interactions. We wish to understand the spatial location of chromosomes in dividing cells in order to understand the relationship between chromosome-chromosome interactions and to further investigate the role of chromosomes and their impact on cell biological behavior. In this study, we explored the relative spatial positional relationships of chromosomes [t(9;22) and t(15;17)] in B-ALL cells by using the three-dimensional DNA fluorescent *in situ* hybridization (3D-FISH) method. The results showed that chromosomes [t(9;22) and t(15;17)] showed relatively stable spatial relationships. The relative stability of the spatial location of chromosomes in dividing cells may be relevant to disease.

Keywords: Chromosome; Human genetics; Chromosome territory; 3D-FISH

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1. Introduction

During interphase, each chromosome occupies a separate nuclear space to form a chromosome territory (CT) [1-3], which has been shown to have non-random radial nuclear distributions [4-6]. The spatial conformation of CTs in the nucleus is non-random [7]. Several studies in recent years have demonstrated a link between the spatial conformation of the genome and some basic biological processes (gene recombination, gene expression, differentiation, etc.) [8,9]. Chromosomes are considered natural units of subdivision of the complete genome. Karyotype abnormalities are often present in several clinical conditions such as hematologic tumors [10,11], malformations [12], infertility [13], etc. An interesting phenomenon is that most of the karyotype abnormalities are single points, which seems to suggest the timing of the karyotype abnormalities. Chromosomes adjacent to each other in the nucleus may be more likely to undergo translocation than those relatively distant from each other, suggesting the relevance of spatial location for the occurrence of karyotypic abnormalities. During mitosis, chromosomes can easily be seen as highly condensed structures in cells [14], but their intracellular location and the spatial relationships between chromosomes are poorly understood. Chromosome structure and arrangement in cells are only well observed in the interphase of cells [15].

Previously, we chose to analyze the data of 66,919 valid karyotype abnormalities in the National Cancer Institute database (April 26, 2017) [15]. We analyzed the karyotype abnormalities occurring between chromosomes in the karyotype abnormality data and found a high frequency of karyotype abnormalities occurring. Plus, many karyotypic abnormalities were isolated or rare. The data collected on karyotypic abnormalities (karyotypic abnormalities between chromosomes) were analyzed. We followed two models for this, a planar model of chromosomes on the equatorial plate of the medium-term cell and a circular or a foveal arrangement.

Fluorescent *in situ* hybridization (FISH) is an analytical detection technique. It obtains information on the status of multiple chromosomes or multiple genes by hybridizing fluorescently labeled nucleic acid probes with nucleic acid sequences and further analyzing the probe signals with the aid of fluorescence microscopy [16]. FISH has the advantages of a short detection cycle, high stability, high sensitivity, and stable probes that can be stored for a long time, etc. It is mainly used clinically for tumor diagnosis, prognosis assessment, and guidance of targeted drug therapy [17]. Three-dimensional (3D)-FISH is a genetic sequence-specific biomarker technique based on the traditional *in situ* hybridization technique [18,19]. It can specifically label specific sequences of genetic material in the nucleus without destroying the cell structure. It is because of this 3D-specific labeling feature that 3D-FISH has been widely used in the study of the 3D spatial conformation of genetic material.

In this study, we stained, scanned, and 3D reconstructed the chromosomes of mid-stage cells using the 3D-FISH technique to observe the spatial state of chromosomes in mid-stage cells and to explore the spatial location of chromosomes within the cells and the spatial relationship between chromosomes. We aimed to further understand the chromosomes and the biological behavior between them.

2. Materials and methods

2.1. Samples

BALL-1 and HL-60 cells were purchased and cultured as recommended by ATCC and DSMZ, respectively. Then, they were selected: karyotype t(9;22) and t(15;17), respectively. Another human BALL-1 cell line (male) was used as a control. The cell lines were all treated as interphase cell lines. Each type of cell line was divided into male and female and was predominantly male. Ethical approval was given by the medical ethics committee of Soochow

University (Ethics Approval Number: 2022-536).

2.2. Cell culture and preparation

All cells were terminated at interphase and were hypotonicized (colchicine was added at 0.05 g/mL for one hour). Subsequently, cells were fixed with 3:1 methanol:acetic acid three times. Conventional filming is to observe the karyotype of chromosomes, which requires cell fragmentation and natural dispersion of chromosomes; while this experiment requires observation of the structure and arrangement of chromosomes in the cells, the structural integrity of the cells and effective dispersion of the cells are required. Therefore, we adjusted the height of the drop film and lowered it by about one-third.

2.3. Probe preparation and selection

The type of probe was a fully stained probe purchased commercially (Creative Bioarray, Shirley, NY, USA). Color crosstalk is an important factor affecting the experimental results (especially when crosstalk is between adjacent chromosomes), four fluorochromes per group were chosen to label the chromosomes. For this reason, we used different schemes to mark different chromosomes with different possible distances as fluorochromes and chromosome corresponding marks based on the results of the preliminary data analysis, reducing the chance of crosstalk between possible adjacent chromosomes. In this study, the corresponding cell lines (target cell lines) were hybridized with the probes separately, while cell lines with normal karyotypes were hybridized separately as controls using two sets of probes. Two sets of chromosomes were selected: Group 1 [t(9; 22), sex chromosomes] and Group 2 [t(15;17), sex chromosomes]. Each chromosome was color-coded for single-color fluorescence separately (**Table 1**).

Table 1. Probe fluorescence color and group

Lable	Abs. (nm)	Em. (nm)	Group 1	Group 2
DAPI/Aqu	405	~470	——	——
Green	488	~510	15	22
Red	543	~570	X	X
DIG	594	~615	17	9
BIOTIN	639	~660	Y	Y

2.4. 3D-FISH

2.4.1. Pretreatment

Frozen sections were removed from the slides stored at -80°C, and the tissues were fixed by immersing the slides in ice-cold 4% paraformaldehyde (PFA) for 15–30 minutes at 4°C. The slides were then rehydrated for 5 minutes each in 100%, 90%, and 70% ethanol, followed by washing in distilled water for 1 minute and in PBS for 5 minutes. Subsequently, the slides were heated in distilled water at 100°C for 15 minutes, treated with pepsin solution for 3–15 minutes at 37°C, and washed in PBS. Finally, the slides were dehydrated by incubating them in pre-cooled 75%, 85%, and 100% ethanol for 1 minute at each concentration, and then air-dried.

2.4.2. Co-denaturing and hybridization

The denatured probe was applied to the slide (10 μL of probe for each slide). A coverslip was immediately applied and sealed with rubber cement. The slides were denatured at 80°C for 5 minutes and hybridized at 37–42°C overnight (~20 hours).

2.4.3. Washing

Rubber cement and coverslips were removed, and to prevent drying, the slides were temporarily kept in washing solution at ambient temperature to facilitate the removal of coverslips. The slides were then immersed in SSC/0.3% NP-40 at 74 ± 1°C for 2–5 minutes, followed by immersion in 2X SSC/0.3% NP-40 at room temperature for 5 minutes. Subsequently, the slides were dehydrated by incubation in 75%, 85%, and 100% ethanol for 1 minute at each concentration. Finally, the slides were air-dried in darkness.

2.4.4. Counterstain

A volume of 20 μL of antifade solution with DAPI was placed on the surface, and a cover glass was positioned over it. Any air bubbles that may have formed were carefully removed.

2.5. Confocal microscopy

2.5.1. Microscopy

Confocal imaging was performed on a Zeiss LSM 980 laser scanning confocal system with a Plan-Apochromat ×63/1.40-NA DIC M27 oil-immersion objective.

2.5.2. Confocal scanning method

The pinhole during all the image acquisition was opened at 1 Airy unit. Laser powers and detector gain were optimized for each sample. An Airyscan II detector was also used for image acquisition. The system was controlled by the ZEN software (Zeiss, blue edition). A 405 nm diode laser was used for DAPI excitation; a 488 nm diode laser was used for green probe excitation; an A 543 nm diode laser was used for red probe excitation; a 594 nm diode laser was used for DIG-labeled probe excitation; a 639 nm diode laser was used for BIOTIN-labeled probe excitation. Between the Red probe and the DIG-labeled probe, Zeiss' unique spectral resolution method was used to eliminate the risk of color crosstalk. Image processing was performed by ZEN Airy scan processing using automatic deconvolution parameters; the 3D visualization of images was processed by the ZEN 3D module, and the most appropriate surface parameters were processed for each sample.

2.5.3. Observation of images

The XY chromosome was used as an anchor point to see the other chromosomes themselves and their spatial location in relation to the XY chromosome.

3. Results

From **Figures 1** to **3**, we can see the relatively stable state of the chromosomes. The chromosome arrangement of different cells of the same cell line of the same set of probe hybridization is relatively stable: the spatial position of the three sets of chromosomes can be seen to be relatively stable. The spatial positions of chromosomes in different

cell lines of the same cell type also have similar spatial position relationships. The spatial position relationships of the chromosomes within the cells observed for each set of probe hybridization for the target cell line and the cell line with normal karyotype are also relatively stable.

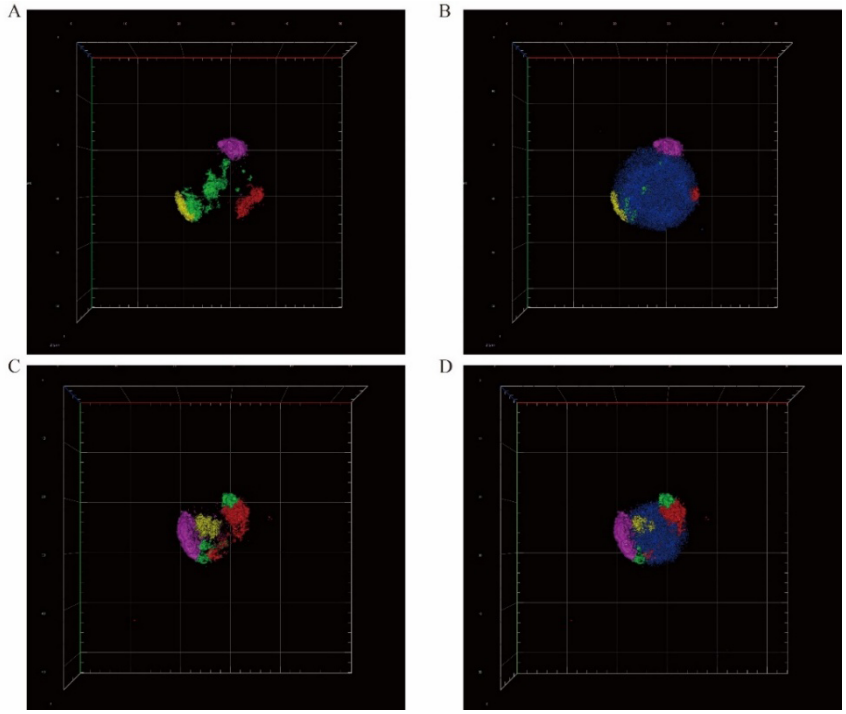

Figure 1. 3D-FISH visualization of chromosomes in normal human (male) nuclei of BALL-1 cell. A, C: chromosome 9 (DIG); 22 (Green); X (Red); Y (BIOTIN); B, D: chromosome 9, 22 with DAPI (Aqu)

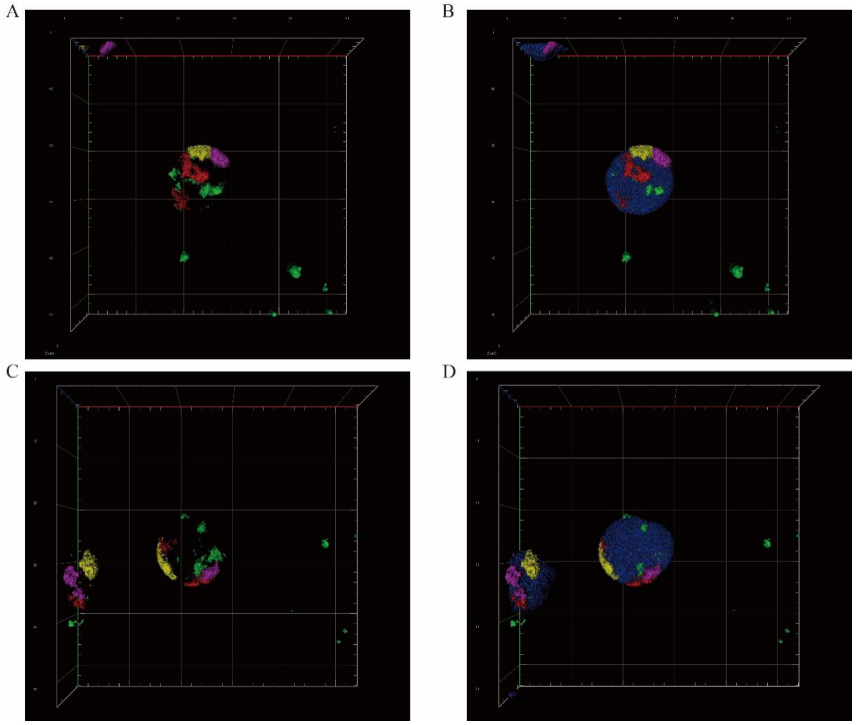

Figure 2. 3D-FISH visualization of chromosomes in case 1's (male) cell nuclei. A, C: chromosome 9 (DIG); 22 (Green); X (Red); Y (BIOTIN); B, D: chromosome 9, 22 with DAPI (Aqu)

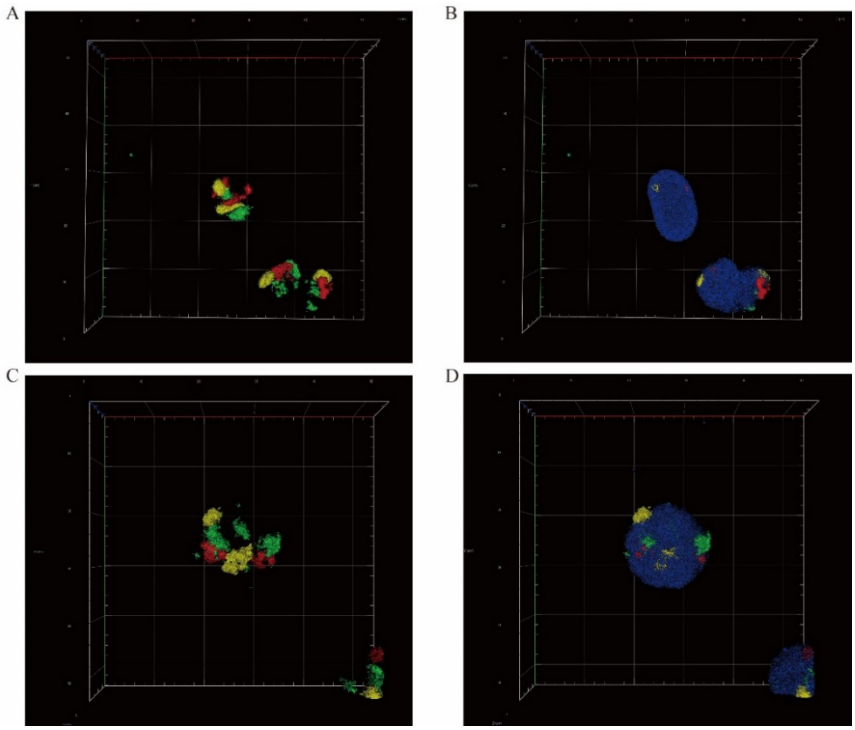

Figure 3. 3D-FISH visualization of chromosomes in case 2's (female) cell nuclei. A, C: chromosome 9 (DIG); 22 (Green); X (Red); Y (BIOTIN); B, D: chromosome 9, 22 with DAPI (Aqu)

4. Discussion

The orientation of the cells, as they drip onto the slide, is random due to the cells being round or oval. The equatorial plates within the cells, and the chromosomes in them, do not maintain a neat and uniform orientation, which makes it somewhat challeging to compare the spatial position of the chromosomes with each other. At the same time, because the cells are affected by the coverslip during the filming process, the structure of the cells is intact but flat, so the chromosomes inside the cells are somewhat squeezed and there is a certain displacement of the spatial position relationship of the chromosomes, which is an overall displacement that does not affect the position relationship. It also does not affect our results and the judgment of the results. Since the cells are approximately round or oval, the cells are filmed by drip film, and the spatial position of the cells currently is random. This has an impact on the processing of information after scanning, especially the confirmation of the spatial position of chromosomes after 3D reconstruction. By adjusting the three-dimensional information of the cells, we can understand their positional relationships more intuitively.

The two sets of probes hybridized different cell lines separately, and both observed relative stability in the spatial positional relationships between chromosomes within the cells. Each set of probes hybridized the target cell line and the cell line with normal karyotype, and their observed spatial position relationship of chromosomes within the cell was also relatively stable. This is a remarkable and meaningful phenomenon. It strongly suggests the stability of the spatial position of chromosomes within the medium-term cell cells. However, we need the support of more data to further refine and enrich our observations.

There are two sets of chromosomes in the cell, X and Y chromosomes as sex chromosomes, and it is very interesting how they are assigned to one of them. More observations are needed to further understand the

implications.

Chromosomes 9 and 22 and chromosomes 15 and 17 are stably present and arranged near each other. This is also true in the same cell line and different cell lines. In the chronic granulocytic leukemia cell line or the normal karyotype cell line, their pattern is consistent. Based on the present phenomena, it appears that the chromosomes may be regularly arranged on the equatorial plate in intermediate cells, as is the case for chromosomes 9 and 22 and chromosomes 15 and 17. However, this requires more data and observations of different probes for further verification.

The relative spatial positions of chromosomes 9, 22, and X and Y, as well as 15, 17, and X and Y, correspond to each other, as the long and short arms of the chromosomes may be in free suspension within the cell and positioned by the chromosome's mitoses. This causes the non-uniform overall position of chromosomes, with small drifts of chromosomes in a relatively stable position. There may be other chromosomes caught in between the individual chromosomes. It is just that they are not marked and displayed for us to observe, thus more experiments are needed to observe and understand this.

The arrangement of chromosomes on the equatorial plate in human intermediate somatic cells is stable and regularly arranged; X and Y chromosomes are arranged correspondingly on the equatorial plate in cells in females, and X chromosomes appear in pairs as sex chromosomes. In male cells, the sex chromosomes are X and Y chromosomes, which are currently seen to appear in proximity. The arrangement of the segregation of X and Y chromosomes needs to be further investigated.

The XX chromosomes are the two pairs of sex chromosomes in females, which naturally belong to two different sides of the equatorial plate of the cell, in a corresponding arrangement. In males, the sex chromosomes are the X and Y chromosomes, and their arrangement is a very interesting expectation. The current observation shows that the position of the X chromosome about the Y chromosome and other groups of chromosomes (15, 17; 9, 22) is also relatively fixed. The prediction is that the two sex chromosomes X and Y may also be arranged in correspondence.

In several cell lines of chronic granulocytic leukemia performed so far, we found that chromosomes 9 and 22, which constitute Ph1, can originate from the same ring or different rings. This is a significant phenomenon, but its clinical significance is not yet clarified, thus we need to do more data accumulation and clinical observation. The translocation of chromosomes 15 and 17 also requires further research.

5. Conclusion

The relative stability of the spatial location of chromosomes in interphase cells may be associated with disease.

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Disclosure statement

The authors declare no conflict of interest.

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