**Proximity within Interphase Chromosome Contributes to the Breakpoint Distribution in Radiation-induced Intrachromosomal Exchanges**

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**BACKGROUND**

Previously, we reported that breaks involved in chromosome aberrations were clustered in several regions of chromosome 3 in human mammary epithelial cells after exposure to either low- or high-LET radiation. In particular, breaks in certain regions of the chromosome tended to rejoin with each other to form an intrachromosomal exchange event. This study tests the hypothesis that proximity within a single chromosome in interphase cell nuclei contributes to the distribution of radiation-induced chromosome breaks. Chromosome 3 in G1 human mammary epithelial cells was hybridized with the multifocal banding in situ hybridization (mBAND) probes that distinguish the chromosome in six differently colored regions, and the location of these regions was measured with a laser confocal microscope. Results of the study indicated that, on a mega-base pair scale of the DNA, the arrangement of chromatin was non-random. Both telomere regions tended to be located towards the exterior of the chromosome domain, whereas the centromere region towards the interior. In addition, the interior of the chromosome domain was preferentially occupied by the p-arm of the chromatin, which is consistent with our previous finding of intrachromosomal exchanges involving breaks on the p-arm and in the centromere region of chromosome 3. Other factors, such as the fragile sites in the 3p21 band and gene regulation, may also contribute to the breakpoint distribution in radiation-induced chromosome aberrations. Further investigations suggest that the 3D chromosome folding is cell type and culture condition dependent.

**MATERIALS AND METHODS**

Human mammary epithelial cells (CH134B6SF5/M10) were cultured to confluence in DMEM medium with supplement of 10% FBS under either simulated microgravity condition or static condition. Peripheral whole blood was collected from a healthy donor in Vacutainer cell tubes containing sodium citrate. Peripheral blood mononuclear cells (PBMCs) were immediately separated by centrifugation, washed twice with PBS. PBMCs at G0 phase and confluent M10 cells (mainly in G1 phase) were fixed in fresh Methanol: Acetic Acid (3:1) fixative. Fixed samples were then washed with 1x PBS without air dry. The samples were immediately hybridized with the XCy5 mBAND metaSystems that distinguish the chromosome in 23 different color bands. Three-dimensional images of each of the six painted regions of chromosome 3 were captured with a Zeiss and a Leica laser confocal microscope (Fig. 1). The captured image was uploaded into Imaris© and Fuji image analysis software. The surface of each of the six chromosome regions was determined (Fig. 1c). The center of each region and the center (not centromere) of the entire chromosome domain were calculated once the surface is defined.

3. The angles extended from the center of each colored region to the adjacent colored regions

The mean angle is the smallest for the regions on the p-arm of the chromosome in comparison to the q-arm. However, the mean angle for the centromere region appeared to be greater than the angles for regions on either the p-or q-arm.

4. Region 2 in intrachromosomal aberrations

Aberrations involving chromosome 3 are associated with a number of known cancer types. Most of breaks involved in intra-chromosome 3 exchanges are found in the middle of the p-arm (Figure 5). The 3p21.3 region (within region 2) is a known fragile site and contains several tumor suppression genes.

**RESULTS**

**4 Gy**

Figure 1. Examples of chromosome 3 of human lymphocytes painted with mBAND (A and B) and chromosome 3 and 6 with FISH (C) after 4 Gy x-ray exposure: A. Chromosomes (C) painted using red arrows (grey arrows indicating normal chromosomes) include translocations, insertion, and deletion.

**RESULTS**

1. Distances between the center of each region and the center of the chromosome domain

The telomere regions (Regions 1 and 6), as well as the q-arm of the chromosome (Regions 4-6) appeared to be located towards the exterior of the chromosome domain. Most of the regions in the p-arm of the chromosome (Regions 1/2, -3), as well as the centromere region (Region 3/4), however, were closer to the center of the chromosome domain.

2. Distances between the center of each region and the center of the chromosome domain

Within the p-arm of the chromosome, the physical distance increased as the genomic distance increased. However, the distance between Region 2 and other regions on the q-arm of the chromosome appeared to be weakly dependent on the genomic separation.

3. The angles extended from the center of each colored region to the adjacent colored regions

The mean angle is the smallest for the regions on the p-arm of the chromosome in comparison to the q-arm. However, the mean angle for the centromere region appeared to be greater than the angles for regions on either the p-or q-arm.

5. Factors that determine the 3D structure of interphase chromosome 3

**CONCLUSIONS**

- On a multi-megabase pair scale of the DNA, the arrangement of chromatin in M10 epithelial cells is non-random.
- In human epithelial cells, both telomere regions tended to be located towards the exterior of the chromosome domain, whereas the centromere region towards the interior. In addition, the interior of the chromosome domain was preferentially occupied by the p-arm of the chromatin, which is consistent with our previous finding of intrachromosomal exchanges involving breaks on the p-arm and in the centromere region of chromosome 3.
- Chromosome conformation is cell type dependent, and is altered in a significant number of cells under various culture conditions, such as simulated microgravity, particularly in the regions close to mid-p arm (3p21), centromere, and 3rd telomere.

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