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 multicolor 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 multi-mega base pair scale of the DNA, the arrangement of centromat 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 chromosome, which is consistent with 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 (CH184BFS5/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 G1 phase and confluent M10 cells (mainly in G1 phase) were fixxed 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 Xcye5 mBAND kit (MetaSystems) which distinct 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.

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 Regions 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.

4. Region 2 in intrachromosomal aberrations
Aberrations involving chromosome 3 are associated with a number of known cancer types. Most of breaks involved in intrachromosomal 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.

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

CONCLUSIONS
- On a multi-megabase pair scale of the DNA, the arrangement of centromat 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 chromosome, 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 cell culture condition dependent manner.

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