Chromatin Folding, Fragile Sites, and Chromosome Aberrations Induced by Low- and High- LET Radiation

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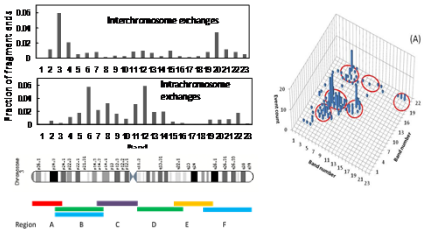
Abstract

We previously demonstrated non-random distributions of breaks involved in chromosome aberrations induced by low- and high-LET radiation. To investigate the factors contributing to the break point distribution in radiation-induced chromosome aberrations, human epithelial cells were fixed in G1 phase. Interphase chromosomes were hybridized with a multicolor banding *in situ* hybridization (mBAND) probe for chromosome 3 which distinguishes six regions of the chromosome in separate colors. After the images were captured with a laser scanning confocal microscope, the 3-dimensional structure of interphase chromosome 3 was reconstructed at multi-mega base pair scale. Specific locations of the chromosome, in interphase, were also analyzed with bacterial artificial chromosome (BAC) probes.

Both mBAND and BAC studies revealed non-random folding of chromatin in interphase, and suggested association of interphase chromatin folding to the radiation-induced chromosome aberration hotspots. We further investigated the distribution of genes, as well as the distribution of breaks found in tumor cells. Comparisons of these distributions to the radiation hotspots showed that some of the radiation hotspots coincide with the frequent breaks found in solid tumors and with the fragile sites for other environmental toxins. Our results suggest that multiple factors, including the chromatin structure and the gene distribution, can contribute to radiation-induced chromosome aberrations.

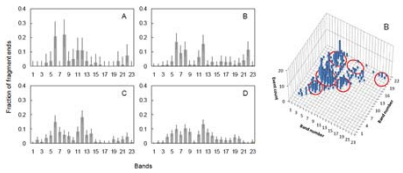
Introduction

Location of breaks participated in inter- and intrachromosome exchanges after gamma exposure

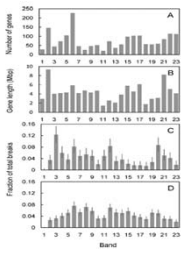


Breaks involved in intrachromosome exchanges are clustered around 3p21, 3q11 and 3q26. Most of the rejoinings occurr between a break in 3p21 and one in 3q11.

Location of Breaks that Participated in Inter- and Intra- Chromosome Exchanges after High LET Radiation



Breakpoint distribution for chromosome fragment ends participating in intrachromosomal exchanges induced by gamma rays of low dose rate (Panel A), neutrons (Panel B), gamma rays of high dose rate (Panel C) or Fe ions (Panel D).



Comparison between the distributions of the genes and of the breakpoints in chromosome 3.

Panel A: Number of protein coding genes in each of the bands;

panel B: total length of protein coding genes in the bands;

panel C: distribution of all breaks for combined low-LET radiations (γ rays of both dose rates);

panel D: distribution of all breaks for combined high-LET radiations (neutrons and iron ions).

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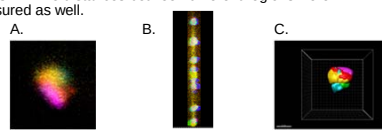
Materials and Methods

Cells
Human epithelial cells (CH184B5F5/M10) were cultured in chamber slides. Upon confluent, the cells in were fixed with methanol/acetic acid.

Interphase chromosome painting with mBAND probes
The interphase cells were hybridized using the *XCyte3* mBAND kit from MetaSystems. The 3-D images of chromosome 3 were captured with a laser scanning confocal microscope, and the three dimensional structure of interphase chromosome 3 with six colored regions was reconstructed using 3D analysis software Imaris7.4. The distances between different regions were measured as well.

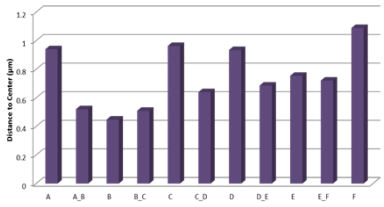


A and B. Images of chromosome 3 acquired using confocal microscopy; C. 3D reconstruction of chromosome 3 using Imaris7.4 module.

The data on the distribution of gene number, gene length, GC content, and fragile sites in various types of cancers, are obtained from various databases.

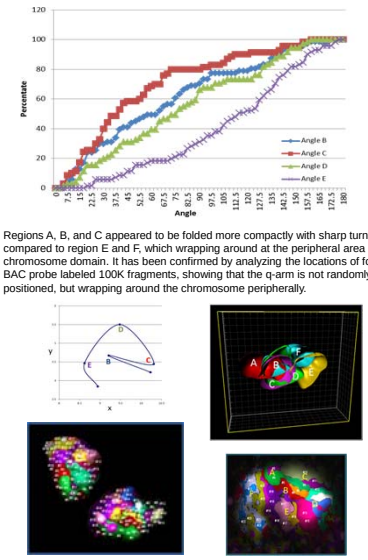
Results

The Distance between Different Regions of Chromosome 3 and the Center of the Chromosome 3D Domain



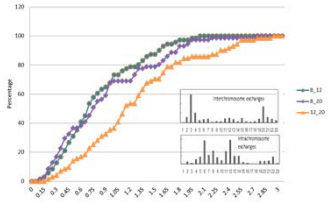
The center of Region B is closest to the center of the domain of chromosome 3. Region A (telomere on the p-arm), region C (close to centromere), and region F (telomere on the q-arm) are father away from the chromosome domain center than either region B or region E.

Distribution of Angles Extended to the Neighboring Regions



Regions A, B, and C appeared to be folded more compactly with sharp turns compared to region E and F, which wrapping around at the peripheral area of the chromosome domain. It has been confirmed by analyzing the locations of four BAC probe labeled 100K fragments, showing that the q-arm is not randomly positioned, but wrapping around the chromosome peripherally.

Distribution of Distance between Regions B, 12, and 20, which Involve in Intra-Chromosome Aberrations



The distance between regions 12 and 20 was much longer than the distance from B to 12, and from B to 20, indicating that regions 12 and 20 may exhibit a lower frequency of intra-chromosome exchanges with each other.

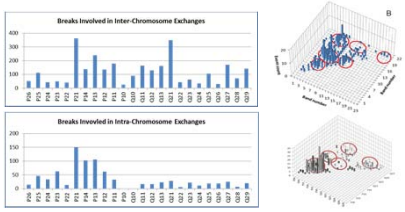
Chromosome 3 and Cancer

Chromosome 3 contains multiple oncogenes and tumor suppressor genes. Chromosome aberrations in the p-arm are known to be associated with various cancer types.

Topography	Case	Case with Ch 3 Aberration	%	Case Analyzed
Bone and Soft Tissues	3406	576	16.9	500
Breast	1128	293	25.9	245
Cadiovascular	48	2	4.2	1
CNS	2511	208	8.3	175
Digestive System	2285	543	23.8	492
Endocrine System	605	115	24.8	107
Female Genital Organs	1552	266	17.1	225
Male Genital Organs	609	94	15.4	63
Respiratory System	1128	365	32.4	319
Skin	331	76	22.9	71
Urinary Tract	2166	640	29.5	569
Overall	15769	3178	20.2	2767

Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (2013). Mitelman F, Johansson B and Mertens F (Ed.), http://cgap.nci.nih.gov/Chromosomes/Mitelman

Location of Breaks that Participated in Inter- and Intra- Chromosome Exchanges in Solid Tumors



The hotspots for radiation exposure coincide with some but not all hotspots for cancers. The region of 3p21.3 (band 6) is a known fragile site and contain several tumor suppression genes.

Conclusions

- The regions towards the telomeres are likely to occupy the peripheral area of the chromosome domain. These heterochromatin regions locating in the peripheral of the chromosome domain may provide the structural support, define the chromosome territory, and maintain the chromosome integrity.
- The present results showed that Band 6-8 tends to locate near the interior of the chromosome domain, and are localized closely to Band 12-13 and Band 19-22. This finding is consistent with the frequency of intra-chromosome exchanges between breaks in these regions.
- The non-random breakpoint distribution in chromosome 3 after radiation exposure may be associated with the folding of chromatin in interphase.
- Other factors, including the location of the fragile sites and transcription activities, may also contribute to the distribution of radiation-induced inter- and intra- chromosome exchange hotspots.
- The distribution of breaks participated in inter- and intra-chromosome exchanges found in chromosome 3 of solid tumors is in partial agreement with radiation induced chromosome exchange hotspots.