FISH and PNA-FISH

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Stochastic effect
• Chromosome DNA is broken by ionizing radiation
  - Induction of DNA breaks
  - Accumulation of Abnormal genes
  - Error in repair

Therapy-related leukemia and chromosome translocations
• t(9;22); Chronic myelocytic leukemia
  - ABL - BCR
  - Alu in the BCR gene
  - A-Bomb survivors (Hiroshima)

• t(15;17); Acute promyelocytic leukemia
  - PML - RARA
  - 3 BCRs in the PML gene
  - 2nd intro of the RARA gene
  - Mitoxantrone, radiation

• 11q23 abnormalities; Therapy-related and infantile leukemias
  - MLL - more than 50 partner genes
  - BCR in the MLL gene
  - Topo II inhibitors (etoposide)

Radiation and cancer
- DNA damage and error in repair
- Cancer
Dicentric chromosome

Error in repair
Induction of DSBs

Ring chromosome

Error
Induction of DSBs

“Dic and Ring” induced by ionizing radiation

Stable and unstable abnormalities

Two models for chromosome translocations

Mechanism of chromosome translocation (1)
Mechanism of chromosome translocation (2)

- Encounter of two specific loci
- Gene positioning (Contact first)
- Induction of DNA double stranded breaks (DSBs)
- Topoisomerase II consensus seq., Alu DNase I hypersensitivity sites?
- Misjoining of DSBs
- No movement of DSBs induced by IScel1 (Misteli et al, 2007)

Chromosome territories

Theodor Boveri, 1888, 1909

Non-radioactive in situ hybridization with chromosome specific DNA probes

Specific detection of more than one hybridization sites in both metaphase spreads and interphase nuclei.

Lichter et al., 1988

FISH and PNA-FISH

1) chromosome
2) FISH
3) PNA-FISH

Fluorescence in situ hybridization (FISH)

- DNA; DNA-FISH
- RNA; RNA-FISH
- Multicolor FISH
- 3D FISH

Light optical serial sectioning and 3D reconstruction of chromosome 18 and 19 in a human lymphocyte

Specific detection of more than one hybridization sites in both metaphase spreads and interphase nuclei.

Cremer C and Cremer T, 2006
Fluorescence in situ hybridization (FISH)

Chromosome DNA

annealing

Hybridization

DNA probe

fluorescence dye

Fluorescence in situ hybridization (FISH)

Gene locus

Chromosome painting


Topological association of the Bach2 gene with the centromere of chromosome 6

DNA-FISH

Ono A., et al; Genes, chromosomes & cancer 2006

Induction of BACH2 gene by imatinib

RNA-FISH

Ono A., et al; Genes, chromosomes & cancer 2006

Multicolor FISH

- M-FISH

- Spectral Karyotyping (SKY) FISH

Garini Y., et al; Bioimaging 1996

24-color 3D FISH


Sun J., et al; PLoS one 2010

Induction of BACH2 gene by imatinib

numbers of signals/cell

Before

After Imatinib treatment

percentage of cells

0      1       2                  0       1

2
Biological dosimetry with chromosome analysis

1) Dicentrics and/or rings analysis (unstable)
2) Chromosome translocation analysis (stable)
1) Premature chromosome condensation (PCC) analysis
1) The Cytokinesis-block micronucleus (CBMN) assay

Gold Standard!!

Chromosome analysis with Giemsa stained samples

1) Culture PBLs with PHA for 48 hrs
2) Fixed with Carnoy
3) Preparation of metaphase spreads
4) Giemsa staining

Good; fast, inexpensive
Not good; difficult

Fluorescence in situ hybridization (FISH) analysis

Chromosome DNA

1) anealing
2) Hybridization

DNA probe

Fluorescence dye

Good; easy
Not good; time consuming, expensive

FISH and PNA-FISH

1) chromosome
2) FISH
3) PNA-FISH

PNA probe

- High binding affinity to its complementary DNA or RNA
- Differentiation of single-base mismatch by high destabilizing
- Stability to nuclease and protease
- Salt independence during hybridization with DNA sequence
- Triplex formation with continuous homopurine DNA

FISH analysis with centromere and telomere PNA probes

1) Culture PBLs with PHA for 48 hrs
2) Fixed with Carnoy
3) Preparation of metaphase spreads
4) Hybridization with centromere and telomere PNA probes
Times required for chromosome analysis

<table>
<thead>
<tr>
<th></th>
<th>Giemsa</th>
<th>FISH</th>
<th>PNA-FISH</th>
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<tbody>
<tr>
<td>Cell culture</td>
<td>48 hrs</td>
<td>48 hrs</td>
<td>48 hrs</td>
</tr>
<tr>
<td>aging</td>
<td>0</td>
<td>&gt; 48 hrs</td>
<td>0</td>
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<tr>
<td>Staining/Hybridization</td>
<td>0.5 hrs</td>
<td>&gt; O/N</td>
<td>1 hr</td>
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</tbody>
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Dicentric chromosome analysis

Dicentric chromosome analysis using FISH with telomere and centromere PNA probes could be useful for biological dosimetry.

The incidence of dicentric chromosomes in the metaphase spreads detected by FISH correlated very well with those by Giemsa analysis except the samples irradiated at 15 Gy. Square root of mean squared error in estimated dose using inverse spline regression using FISH analysis with dicentric chromosomes was lower than that using Giemsa analysis (0.391 vs 0.791, respectively) - Shi L., et al; Rad Res 2012.

Multi-centromeric chromosomes formed after ionizing irradiation

Multicentromeric chromosomes could be more frequently missed in Giemsa than FISH analysis.

The incidence of multi-centromere chromosomes detected by Giemsa analysis failed to show linear correlation with the irradiation dose. - Shi L., et al; Rad Res 2012

Ring chromosomes formed after ionizing irradiation

Ring chromosomes could be more frequently missed in Giemsa than FISH analysis.

The incidence of ring chromosomes detected by Giemsa analysis failed to show linear correlation with the irradiation dose. - Shi L., et al; Rad Res 2012

Dic + Ring chromosomes after ionizing irradiation

Since the mean squared error of the estimated irradiation doses is smallest using dic + ring in both FISH and Giemsa analysis, dic + ring could be a good index for the estimation of radiation doses.

The mean squared error of the estimated irradiation doses of samples irradiated at higher dose with dic + ring (0.337 and 0.590 by FISH and Giemsa, respectively) were smaller compared to those of dicentrics (0.391 and 0.791 by FISH and Giemsa, respectively). - Shi L., et al; Rad Res 2012

The frequency of chromosomal aberrations (dicentrics and rings) is a linear-quadratic function of dose.

At low doses, both breaks may be caused by the same electron; the probability of an exchange aberration is proportional to dose (D). At higher doses, the two breaks are more likely to be caused by separate electrons. The probability of an exchange aberration is proportional to the square of the dose (D²).

From Radiology for the Radiologist 7th ed, Eric J. Hall, Armas J. Garcia.
Uncertainty of dose estimation
If 25/500 cells carry one dicentric chromosome,

Numbers of Double strand breaks
(associated with radiation dose)

Misjoining of DNA ends
(Accuracy of DNA repair)

Factors affecting chromosome abnormalities

Difference among individual?

More resolution and more speed!

(Modified from Cytogenetic analysis for Radiation Dose Assessment, A Manual, IAEA 2001)