Overview

Radiation damage to cells
- DNA

Effects of radiation damage on cells
- Cell cycle arrest
- DNA repair
- Cell death / apoptosis

Detecting radiation damage
- Cytogenetic assays
- Protein phosphorylation
- Changes in gene expression
- Changes in cellular metabolism

Radiation causes cellular damage
Ionizing radiation removes electrons from matter, causing molecular bonds to break.

- Radiation damage can occur throughout the cell
- Signaling cascades communicate radiation damage
Radiation causes cellular damage

DNA damage is the most critical. Need DNA to make everything else in the cell.

Types of radiation DNA damage

- Abasic Site
- Oxidative Base Damage

Types of DNA damage cont.

- Double-strand breaks are thought to be responsible for most cell killing due to ionizing radiation
- Double-strand Break (DSB)
- Single-strand Break (SSB)
Cells can detect DSB

The MRN complex (Mre11, Rad50, Nbs1) recruits and activates ATM, which initiates damage signaling and DNA repair.

Ku70/80 also binds broken DNA ends, activates DNA-PKcs
Recruits other proteins to signal damage and initiate repair of the break.

Signaling from damage

Some common p53-activated genes
The mammalian cell cycle

Cyclins: made and degraded each cell cycle
Cyclin-dependent kinases: drive cell division

Radiation exposure triggers checkpoints that halt cell cycle progression.

G1 arrest

DNA damage

Wild-type
p53-/- or p21-/-

p21 binds to G1 cyclin/cdk complexes and inhibits kinase activity
Arrest can be transient or permanent

Repair of DSB

Homologous recombination
Non-Homologous End joining (NHEJ)

Most accurate
Most common
**Incorrect repair - mutation**

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>Spont.</th>
<th>2 Gy gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>point</td>
<td>2.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Partial gene del.</td>
<td>2.2</td>
<td>8</td>
</tr>
<tr>
<td>Total gene del.</td>
<td>0.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Multiple loci del.</td>
<td>0.05</td>
<td>5.5</td>
</tr>
</tbody>
</table>

**Incorrect repair - cytogenetic damage**

- **Translocation**: not lethal, but may activate an oncogene
- **Dicentric and fragment**: usually lethal

**Translocation in Chronic Myeloid Leukemia**
Multiplex FISH

Paint each chromosome a different color

FITC  SPECTRUM O  TEXAS RED  Cy5  DRAQ

Combined

“Two break” stable aberrations

Inter-arm (translocation)

mFISH

“Two break” stable aberrations

Inter-arm (pericentric inversion)

mBAND
**Intra-arm (paracentric inversion)**

- **Inversion**
  - **Joining**

**"Two break" stable aberrations**

**Cell killing - clonogenic survival**

- **Survival**
  - $10^0$
  - $10^{-1}$
  - $10^{-2}$
  - $10^{-3}$

**Radiation survival curves**

- **Survival vs. Dose (Gy)**
  - Repair deficient cells
Low dose-rate protects cells

Cell killing by radiation

- **Apoptosis**
  Complex genetic program triggering cellular "suicide," or "programmed cell death."

- **Necrosis**
  Rapid depletion of ATP, breakdown of cell membrane, inflammation, nuclei shrink and condense, random degradation of DNA

- **Mitotic catastrophe**
  Abnormal mitosis with cytogenetic damage, conflicting signals, checkpoint failure

Hallmarks of apoptosis

- Chromatin condensation
- Phosphatidylserine translocates from inner to outer cell membrane
- Loss of mitochondrial membrane potential
- Caspase activation, protein cleavage
- DNA laddering - nucleosome fragments
Application to Biodosimetry

Cellular responses to radiation provide opportunities for biodosimetry.
- The larger the dose, the greater the biological response

Needed in the event of large-scale radiological event
- Medical Triage
- Active reassurance - reduce panic

Detection of radiation damage to cells can be translated into an estimate of exposure
- Cytogenetics
- Protein phosphorylation
- Gene expression
- Metabolic changes

Cytogenetics - Dicentrics

Assayed in peripheral lymphocytes
**Cytogenetics - Dicentrics**

"Gold standard" for radiation biodosimetry
- Specific for radiation damage
- Stable to about 6 months after exposure
- Informative for doses 0.2-5 Gy
- Used for biodosimetry in many accidents (Chernobyl, Goiânia, Istanbul, Bangkok etc.)

**Cytogenetics - Micronuclei**

Simpler assay with great automation potential
- Stable to about 6 months after exposure
- Informative for doses 0.3-5 Gy
- International standards for scoring

**Cytogenetics - PCC**

Premature Chromatin Condensation
- Informative for doses 0.2-10 Gy
- Potential for automation
- Without cell division
  - Requires fusion with mitotic cells to force condensation of chromatin
- With cell division
  - Condense chromosomes using Calyculin A
Protein phosphorylation

**Phospho-γH2AX forms foci in irradiated cells**
- Linear over broad dose range
- Informative for first day after exposure
- Can be automated for high-throughput
- Does not require cell division

Rothkamm & Lobrich (2003)
PNAS 100:5057

Gene expression

**Potential new approach**
- Informative for doses 0.2 - 8 Gy
- Useful in first 2-3 days after exposure
- Specificity for radiation needs testing

Amundson et al., (2000)
Radiation Research, 154 (3): 342-346

Screening with microarrays allows rapid discovery of potential radiation exposure markers
**Gene expression**

Advanced nanofluidics are being developed for self-contained “biochips” for rapid radiation dose assessment in emergencies.

**Metabolomics**

**Potentially most rapid and least invasive**
- Cellular changes in response to radiation result in changes in metabolism
- Results in changes in small molecules secreted in urine, saliva, sweat etc.
- Specificity for radiation specificity and dose dependence need testing

Before RT
Immediately after RT
24 hours after RT

- Urine
- Sweat
- Saliva

**Metabolomics**

Marker discovery and testing using UPLC-MS(ToF)

Current technology could easily be adapted to rapidly screen for a radiation signature
Summary of biological effects

- Radiation causes damage to all cellular molecules, but DNA damage is most critical.
- DNA damage starts signaling cascades that result in:
  - Cell cycle arrest
  - DNA repair
  - Apoptosis or other cell death
- Radiation damage can be detected by:
  - Cytogenetics
  - Changes in gene expression
  - Changes in protein expression or phosphorylation
  - Changes in metabolic products