Molecular Mechanisms of DNA and Chromosome Damage and Repair

Chapter 2

Eric J. Hall, Amato Giaccia,
Radiobiology for the Radiologist

Introduction

• DNA is implicated to be the principal target for the biologic effects of radiation
• The damage is produced through breakage of molecular bonds by interaction with either fast electrons or free radicals
• Depending on the type of the damage it could be lethal to the cell or can be repaired (sub-lethal damage)

Structure of DNA

• DNA - deoxyribonucleic acid is a polymer with the monomer units of nucleotides
• There are four different types of nucleotides in DNA, differing only in the nitrogenous base: adenine (A), guanine (G), cytosine (C), thymine (T)

Structure of DNA

• DNA molecule has many deoxyribo-nucleotides (bases) linked in a chain-like arrangement
• Molecule is wound into a double-helix structure
• Bases are held by hydrogen bonds and are paired complimentary into a double-strand structure:
  – adenine with thymine (A-T)
  – cytosine with guanine (C-G)

Structure of DNA

• Each half is a template for reconstruction of the other half
• During cell division each strand is self-replicated resulting in identical molecules

Chromosomes

• DNA molecules carry the genetic information
• Chromosome is an organized structure of DNA and DNA-bound proteins (serve to package the DNA and control its functions)
• Chromosomes are located mostly in cell nucleus (some amount is in mitochondria)
The human genome has ~3 billion base pairs of DNA arranged into 46 chromosomes.

Chromosomes:
- Chromatid – one of the two identical parts of the chromosome after S phase
- Centromere – the point where the two chromatids touch, and where the microtubules attach (anaphase)
- Short arm
- Long arm

Chromosomes can be viewed with light microscope during the cell division phase, when stained with a dye. Each appears to have distinct ‘bands’.

Indirect action is dominant for sparsely ionizing radiation (x-rays).

Radiation damage to DNA:
- Direct action: a secondary electron interacts with the DNA
- Indirect action: the secondary electron interacts with a water molecule to produce a hydroxyl radical (OH).
  - About 2/3 of the x-ray damage to DNA is caused by the OH.
- The DNA helix has a diameter of ~2 nm; free radicals produced in a cylinder with a diameter ~4 nm can affect the DNA.

Radiation damage to DNA:
- A: Two-dimensional representation of the normal DNA helix
- B: A break in one strand is of little significance because it is repaired using the opposite strand as a template
- C: Breaks in both strands, if separated, are repaired as independent breaks
- D: If breaks occur in both strands and are directly opposite or separated by only a few base pairs, this may lead to a double-strand break in which the chromatin snaps into two pieces
Characteristic distances

- Energy is absorbed along the tracks of charged particles, producing multiple damage
- Radiation chemistry terminology:
  - Blob: 100 to 500 eV energy, ~12 ion pairs, 7 nm diameter
  - Spur: up to 100 eV energy, ~3 ion pairs, 4 nm diameter
- Short track

For \( x \)- and \( y \)-rays 95% of energy is deposited in spurs
For \( \alpha \)-particles and neutrons – mostly in blobs

Measuring DNA strand breaks

- Both single-strand and double-strand DNA breaks can be measured readily
- Agarose gel electrophoresis
  - DNA is negatively charged, moves in electrical field
  - The DNA is isolated from irradiated cells and the pieces are passed through a porous filter or a gel
  - Can quantify induction and repair of breaks

- Measuring DNA strand breaks

- Agarose gel electrophoresis
  - Add enough electrophoresis buffer to cover the gel
  - Each well in the gel is filled with buffer solution

Agarose gel electrophoresis

- Example: PFGE (pulsed-field gel electrophoresis) with break repair mechanism suppressed by putting sample on ice
- The larger the dose, the more the DNA is broken up into smaller pieces
- Smaller pieces move faster and farther

Measuring DNA strand breaks

- Example: single cell electrophoresis (comet assay)
- In an intact cell DNA does not migrate after lysis
- Fragmented DNA in irradiated sample resembles a comet (stained with a dye binding to DNA)
- By changing the pH of lysis solution can observe either SSB or DSB

Measuring DNA strand breaks

- More recent technique: radiation-induced foci assay
  - Signaling and repair proteins localize near strand breaks
  - Stained for DNA-damage sensing proteins
  - Staining showing the location of nuclei

Measuring DNA strand breaks

- Example: PFGE (pulsed-field gel electrophoresis) with break repair mechanism suppressed by putting sample on ice
- The larger the dose, the more the DNA is broken up into smaller pieces
- Smaller pieces move faster and farther
**Experimental evidence**

- A dose of radiation that induces an average of one lethal event per cell leaves 37% still viable.
- For this dose (1-2 Gy) the number of DNA lesions per cell detected immediately:
  - Base damage > 1,000; Single-strand breaks ~1,000
  - Double-strand breaks ~40
- Cell killing does not correlate at all with single-strand breaks, which can be easily repaired.
- It relates better to double-strand breaks, due to induced chromosome aberrations.

**DNA repair pathways**

- Mammalian cells have developed a number of specialized pathways to sense and repair DNA damage.
- Depending on a type of damage (base damage, SSB, DSB, sugar damage, crosslinks) different mechanisms are invoked.
- Stage of a cell cycle also affects these pathways.

**The cell cycle**

- **M** - mitosis, identifiable by light microscopy and the most constant time (~1 hr).
- **S** - DNA synthesis phase.
- **G₀** - the first gap in activity, between mitosis and the S phase (most variable length).
- **G₂** - the second gap in activity, between S phase and the next mitosis.
- If the cells stop progressing through the cycle (if they are arrested) they are in **G₀**.

**Cell mitosis**

- Cell mitosis goes through several phases:
  - Interphase: cell growth; near the end, the chromosomes of the cell duplicate in preparation for cell division.
  - Prophase: the chromosomes coil, becoming short and thick; the spindle fibers attach to the centromeres of the chromosomes and to both ends of the cell.
  - Metaphase: all of the chromosomes line up across the cell center.
  - Anaphase: the chromosomes separate, one copy of each is pulled to each end of the cell by the spindle fibers.
  - Telophase: a new nuclear membrane forms in each daughter cell.
Radiation-induced aberrations

Lethal aberrations include dicentrics (A), rings (B), and anaphase bridges (C).

Radiation-induced aberrations

A: Symmetric translocation: radiation produces breaks in two different pre-replication chromosomes. The broken pieces are exchanged between the two chromosomes, and the “sticky” ends rejoin.

B: Deletion: radiation produces two breaks in the same arm of the same chromosome. Symmetric translocations and small deletions are nonlethal.

Radiation-induced aberrations

• Formation of a deletion by ionizing radiation in an interphase chromosome.
• After two breaks occur in such a way as to isolate a loop of DNA, the “sticky” ends rejoin, and the deletion is lost at a subsequent mitosis because it has no centromere (may include the loss of a tumor suppressor gene, leading to cancer).

Radiation-induced aberrations

• Frequency of chromosomal aberrations (dicentrics and rings) is a linear-quadratic function of dose because the aberrations are the consequence of the interaction of two separate breaks.
• At low doses, both may be caused by the same electron; the probability of an aberration is $\sim D$.
• At higher doses, the two breaks are more likely to be caused by separate electrons; the probability of an exchange aberration is $\sim D^2$.

Introduction

• A cell survival curve describes the relationship between the radiation dose and the proportion of cells that survive.
• “Survival” could have different meanings, e.g. if cell is not capable to divide - it did not survive (mitotic death).

Cell Survival Curves

Chapter 3

Eric J. Hall., Amato Giaccia, Radiobiology for the Radiologist
Mechanisms of cell death after irradiation

- The main target of radiation is cell’s DNA; single breaks are often reparable, double breaks lethal
- Mitotic death – cells die attempting to divide, primarily due to asymmetric chromosome aberrations; most common mechanism
- Apoptosis – programmed cell death; characterized by a predefined sequence of events resulting in cell separation in apoptotic bodies
- Bystander effect – cells directly affected by radiation release cytotoxic molecules inducing death in neighboring cells

Cell survival curves

- The capability of a single cell to grow into a large colony is a proof that it has retained its reproductive integrity
- Cell survival curves usually are presented in the form with dose plotted on a linear scale and surviving fraction on a log scale
- Straight-line dependence means that the surviving fraction is an exponential function of dose
- At higher doses the curve bends

Cell survival curve: multi-target model

- Multi-target single hit model: assume the cell has \( n \) targets to be ‘hit’ for the cell to not survive
- Probability of each ‘hit’ not being successful is \( e^{-D/D_0} \)
- Probability of each ‘hit’ being successful is \( 1 - e^{-D/D_0} \)
- Probability of all \( n \) targets within a cell to be ‘hit’ is \( (1 - e^{-D/D_0})^n \)
- The probability of survival of cell containing \( n \) targets:

\[
S = \frac{N}{N_0} = 1 - (1 - e^{-D/D_0})^n
\]

Cell survival curve parameters

**Multi-target model**

- \( D_1 \) – initial slope (the dose required to reduce the fraction of surviving cells to 37% of its previous value); \( D_0 \) – final slope
- \( D_q \) – quasi-threshold, the dose at which the straight portion of the survival curve, extrapolated backward, cuts the dose axis drawn through a survival fraction of unity
- \( n \) – extrapolation number
- Radiosensitive cells are characterized by curves with steep slope \( D_0 \) and/or small shoulder (low \( n \))

Cell survival curve parameters

**Multi-target model**

\[
S = 1 - \left(1 - e^{-D/D_0}\right)^n
\]

for \( D \gg D_0 \)

\[
S = ne^{-D/D_q}
\]

- Setting \( D=0 \), find \( n \) – the number of targets
- To find \( D_q \) set \( S=1 \)
- Relationship between \( n \) and \( D_q \):

\[
1 = ne^{-n/D_q}, \quad D_q = D_q \ln n
\]
Survival curves and LQ model

- **Linear-quadratic (LQ) model** assumes there are two components to cell killing, only two adjustable parameters
- No final straight portion that is observed experimentally
- An adequate representation of the data up to doses used as daily fractions in clinical radiotherapy

\[ S = \frac{N}{N_0} = e^{-\alpha D - \beta D^2} \]

**α/β ratios**

- If the dose-response relationship is adequately represented by LQ-model:
  \[ S \sim e^{-\alpha D - \beta D^2} \]
- The dose at which \( \alpha D = \beta D^2 \), or \( D = \alpha/\beta \)
- The \( \alpha/\beta \) ratios can be inferred from multi-fraction experiments
- The value of the ratio tends to be
  - larger (~10 Gy) for early-responding tissues
  - lower (~2 Gy) for late-responding tissues

Cell radiosensitivity

- There is a number of factors that influence cell radiation sensitivity even in vitro (position in cell cycle, genetic abnormalities, environment)
- The mechanism of cell death is different, dominated by apoptosis or mitosis; most cells are in-between
- Dose-response relationship can be described as:
  \[ S \sim e^{-\left( (\alpha_D + \alpha_A) \cdot D - \beta_D \cdot D^2 \right)} \]
  \( M \) - mitotic
  \( A \) - apoptotic
Cell radiosensitivity

- Cells in mitosis (∗) show the same sensitivity as apoptotic cells
- The DNA laddering is indicative of cell apoptosis

Genetic control of radiosensitivity

- A number of genes is involved in determining radiosensitivity of mammalian cells
- In many cases this sensitivity has been related to the greatly reduced ability to repair double-strand DNA breaks
- Some of the inherited human syndromes are associated with high radiosensitivity
  - Ataxia telangiectasia (AT), Down's syndrome, etc.

Radiosensitivity of mammalian cells vs. microorganisms

- Mammalian cells are more radiosensitive mainly due to their larger DNA content
- Simpler microorganisms are resistant up to very high doses

Multi-fraction regimen

- Because multi-fraction regimens are used most often in clinical radiotherapy, it is frequently useful to think in terms of an effective survival curve
- If a radiation dose is delivered in a series of equal fractions, separated by sufficient time for repair of sublethal damage between doses, the effective dose-survival curve becomes an exponential function of dose

$$ S = \frac{N}{N_0} = e^{-\frac{D}{D_0}} $$

Multi-fraction regimen

- The $D_0$ of the effective survival curve, defined to be the dose required to reduce the fraction of cells surviving to 37% ($e^{-1}=0.37$), has a value close to 3 Gy for cells of human origin
- This is an average value and can differ significantly for different tumor types

Calculations using cell survival curves

- $D_0$ is the dose required to reduce the fraction of cells surviving to 37% ($e^{-1}=0.37$)
- The dose to kill 90% of the cell population $D_{10}$ is often used in calculations:
  $$ D_{10} = \ln(10)D_0 = 2.3D_0 $$
Calculation of tumor cell kill
Example 1

• A tumor consists of $10^8$ clonogenic cells. The effective dose-response curve, given in daily dose fractions of 2 Gy, has no shoulder and a $D_0 = 3$ Gy. What total dose is required to decrease number of cells to 1?

$$D_{\text{total}} = \ln 10 \times D_0 = 2.3 \times 2.3 = 5.2 \text{ Gy}$$

The total dose to reduce the cell population to 1 cell ($1/10^8=10^{-8}$, by 8 decades of cell killing) is:

$$8 \times 6.9 = 55.2 \text{ Gy}$$

Calculation of tumor cell kill
Example 2

• Suppose that, in the previous example, the clonogenic cells underwent three cell doublings during treatment. About what total dose would be required to achieve the same probability of tumor control?

• Three cell doublings would increase the cell number by $2^3 = 8$

• Consequently, about one extra decade of cell killing would be required, corresponding to an additional dose of 6.9 Gy. Total dose is $55.2 + 6.9 = 62.1$ Gy.

Summary

• DNA DSBs - the most lethal of ionizing radiation induced damage
• Defective repairs lead to chromosome aberrations, some are lethal
• Cell survival curves are used to characterize the effect of radiation (dose-response relationship)
• Multi-target model and LQ model are the most popular for description; parameters are used to find equivalent treatments