Molecular Mechanisms of DNA and Chromosome Damage and Repair

Chapter 2

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Radiobiology for the Radiologist

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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)

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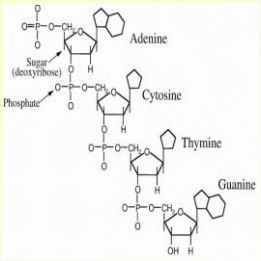
Structure of DNA



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

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Structure of DNA

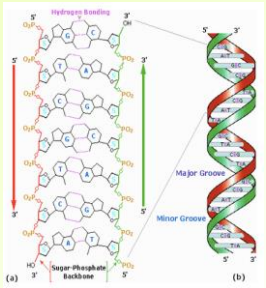

Image from: https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/nucacids.htm

- Molecule is wound into a double-helix structure, one turn of the helix is achieved in 360º
- The diameter of the helix is virtually constant over the entire length and is 1.8nm; the pitch per helix turn (identity period) is 3.37 nm; there are 10 bases per pitch in one strand (0.34 nm apart)

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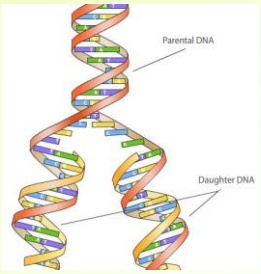
Structure of DNA



- The nucleotides (bases) linked in a chain-like arrangement
- Each half is a template for reconstruction of the other half
- During cell division each strand is self-replicated resulting in identical molecules

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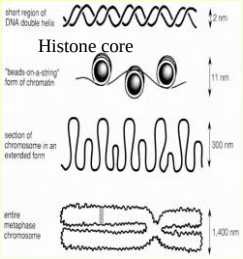
Chromosomes



- 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)

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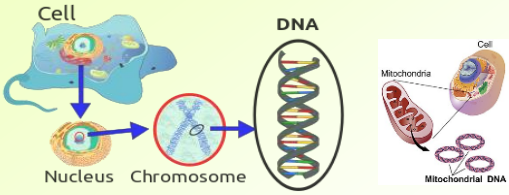
Location of DNA



Images from https://en.wikipedia.org/wiki/DNA, https://en.wikipedia.org/wiki/Mitochondrial_DNA

- The human genome has ~3 billion base pairs of DNA arranged into 46 chromosomes (23 pairs, one chromosome from each parent)
- Mitochondrial DNA in humans have circular chromosome (maternally inherited), 11–28kbp of genetic material

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Chromosomes

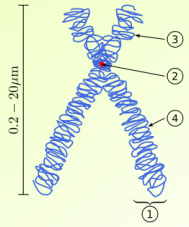

Diagram of a replicated and condensed metaphase eukaryotic chromosome

(1) Chromatid – one of the two identical parts of the chromosome after S phase
(2) Centromere – the point where the two chromatids touch, and where the microtubules attach (anaphase)
(3) Short arm
(4) Long arm

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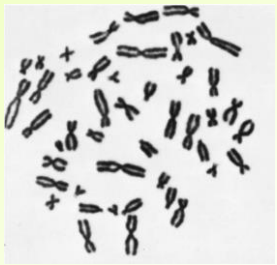
Chromosomes



- Chromosomes can be viewed with light microscope during the cell division phase, when stained with a dye
- Each appears to have distinct 'bands'
- 46 chromosomes (23 pairs) in a human cell

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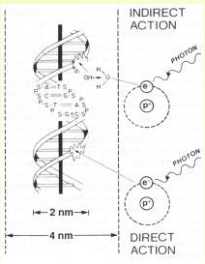
Radiation damage to DNA



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

- 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

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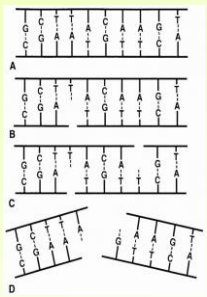
Radiation damage to DNA



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

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Radiation damage to DNA

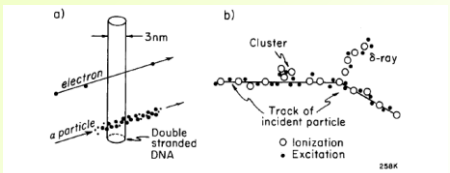

Figure 17-1. (a) Schematic representation of DNA and the tracks of an electron and α particle through it. The fast electron is depicted as depositing energy at 0.25 keV/μm, while the α particle of 5 MeV is shown depositing energy at the rate of 100 keV/μm. (b) Schematic drawing of a charged particle track illustrating the track, ion clusters, and δ ray spurs.

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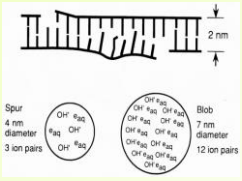
Characteristic distances



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

• For x- and γ-rays 95% of energy is deposited in spurs
• For α-particles and neutrons – mostly in blobs

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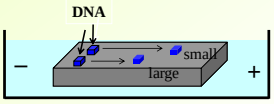
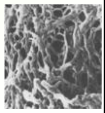
Measuring DNA strand breaks

- Both single-strand and double-strand DNA breaks can be measured readily
- Agarose gel electrophoresis
  - DNA is negatively charged, pieces 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



• Polymerized agarose is porous, allowing for the movement of DNA

Scanning Electron Micrograph of Agarose Gel (1×1 μm) →



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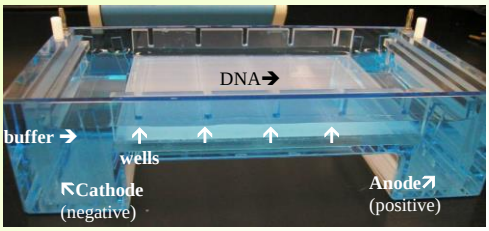
Agarose gel electrophoresis



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

(from *www.rochester.edu/.../Gel%20Electorphoresis%20Lecture%202006.ppt)*

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Measuring DNA strand breaks

DNA laddering

- 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
- Animation link: https://www.youtube.com/watch?v=vtxb6Tr8Y3s

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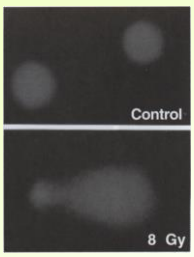
Measuring DNA strand breaks



- 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

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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

Stain showing the location of nuclei

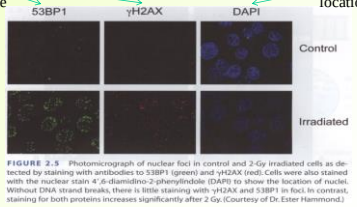

FIGURE 2.5 Photomicrograph of nuclear foci in control and 2-Gy irradiated cells as detected by staining with antibodies to 53BP1 (green) and γH2AX (red). Cells were also stained with the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI) to show the location of nuclei. Without DNA strand breaks, there is little staining with γH2AX and 53BP1 in foci. In contrast, staining for both proteins increases significantly after 2 Gy. (Courtesy of Dr. Ester Hammond.)

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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

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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

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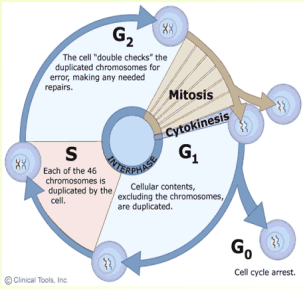
The cell cycle



- **M** - mitosis, identifiable by light microscopy and the most constant time (~ 1 hr)
- **S** - DNA synthesis phase
- **G_1** - the first gap in activity, between mitosis and the S phase (most variable length)
- **G_2** - 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 **G_0**

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DNA repair pathways

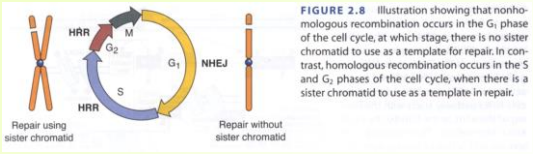

FIGURE 2.8 Illustration showing that nonhomologous recombination occurs in the G_1 phase of the cell cycle, at which stage, there is no sister chromatid to use as a template for repair. In contrast, homologous recombination occurs in the S and G_2 phases of the cell cycle, when there is a sister chromatid to use as a template in repair.

- DSB breaks (most lethal) are repaired by homologous recombination repair (HRR) or nonhomologous end joining (NHEJ) mechanisms depending on the phase of cell cycle
- HHR provides more reliable repair, but errors are possible in both mechanisms

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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

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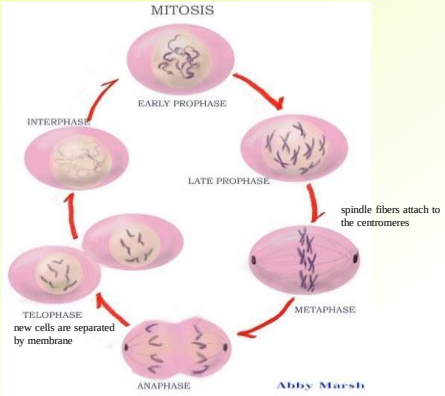

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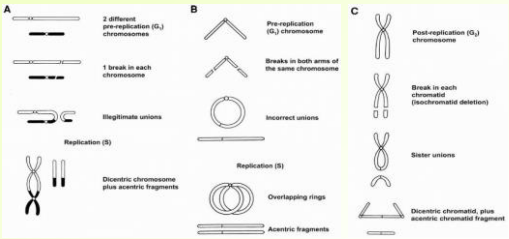
Radiation-induced aberrations



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

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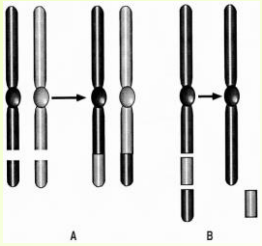
Radiation-induced aberrations



Symmetric translocations and small deletions are nonlethal

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

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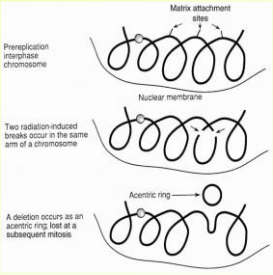
Radiation-induced aberrations



- 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)

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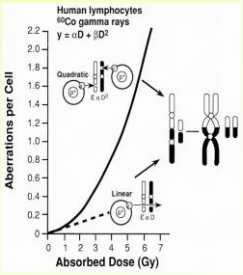
Radiation-induced aberrations



- 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 ~D
- At higher doses, the two breaks are more likely to be caused by separate electrons; the probability of an exchange aberration is ~D^2

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Cell Survival Curves

Chapter 3

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Radiobiology for the Radiologist

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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 a cell is not capable to divide - it did not survive (mitotic death)

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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 (abscopal) effect – cells directly affected by radiation release cytotoxic molecules inducing death in neighboring cells

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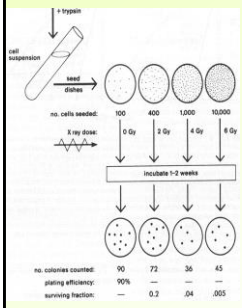
Cell survival curves



- 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

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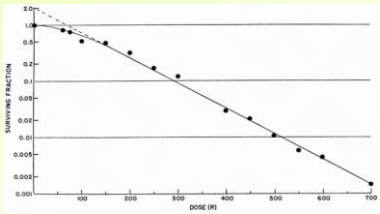
Cell survival curves



• Survival curve for HeLa cells in culture exposed to x-rays
• All mammalian cells, normal or malignant, regardless of their species of origin, exhibit similar x-ray survival curves

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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 $e^{-D/D0}$
- Probability of each 'hit' being successful is $1-e^{-D/D0}$
- 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:

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

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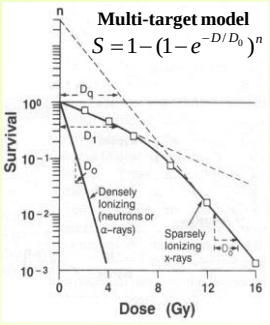
Cell survival curve parameters



- 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)

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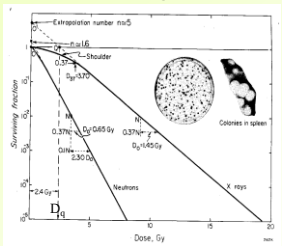
Cell survival curve parameters

Multi-target model



$$S=1-(1-e^{-D/D_0})^n$$

for $D >> D_0$

$$S=ne^{-D/D_0}$$

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

$$1=ne^{-D_q/D_0},\ D_q=D_0\ln n$$

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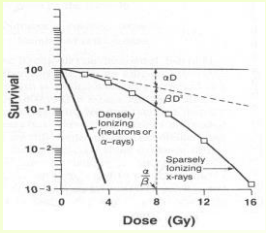
Survival curves and LQ model



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

- **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

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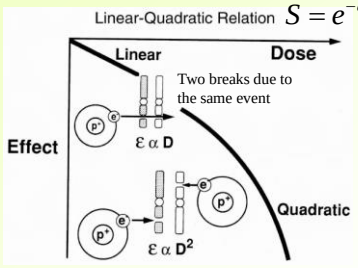
Survival curves and LQ model



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Survival curves and LQ model

- Parameter α reflects intrinsic radio-sensitivity of cells, defining how many logs (base e) are killed or sterilized per Gray in a non-repairable way
- Parameter β represents a repairable portion of damage, requiring ~6 hours for complete repair
- When radiation is delivered in multiple fractions the initial portion (shoulder) of the curve is repeated (providing that fractions are separated by time interval long enough for complete repair of sublethal damage)

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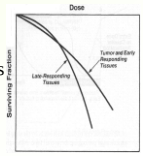
α/β ratios

- 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 α/β 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



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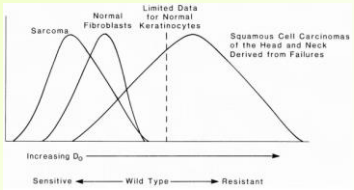
Cell radiosensitivity

Summary of D_0 values for cells of human origin (in vitro studies)



- Cells from human tumors have a wide range of radiation sensitivities
- In general, squamous cell carcinoma cells are more resistant than sarcoma cells

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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:

$$S \sim e^{-(\alpha_M + \alpha_A)D - \beta_M D^2}$$

M – mitotic
A – apoptotic

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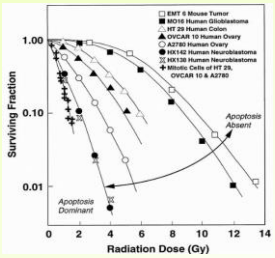
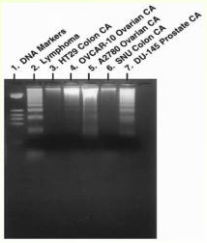
Cell radiosensitivity



Cells in mitosis (+) show the same sensitivity



The DNA laddering is indicative of cell apoptosis

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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 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.

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Radiosensitivity of mammalian cells vs. microorganisms

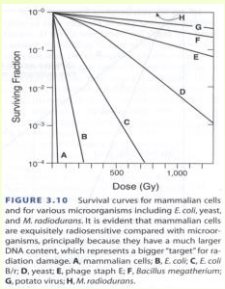

FIGURE 3.10 Survival curves for mammalian cells and for various microorganisms including *E. coli*, yeast, and *M. radiodurans*. It is evident that mammalian cells are exquisitely radiosensitive compared with microorganisms, principally because they have a much larger DNA content, which represents a bigger "target" for radiation damage. **A**, mammalian cells; **B**, *E. coli*; **C**, *E. coli* B/r; **D**, yeast; **E**, phage staph E; **F**, *Bacillus megatherium*; **G**, potato virus; **H**, *M. radiodurans*.

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

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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

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

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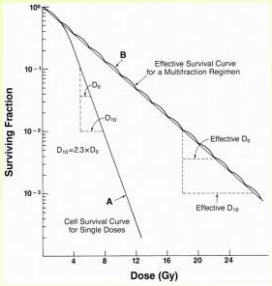
Multi-fraction regimen



- 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

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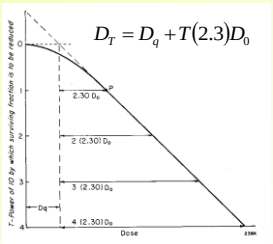
Calculations using cell survival curves



T – number of decades by which the surviving factor is to be reduced

- 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: $D_{10}=\ln(10)D_0=2.3D_0$

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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?

$$D_{10} = \ln 10 \times D_0 = 2.3 \times D_0 = 2.3 \times 3 = 6.9 Gy$$

The total dose to reduce the cell population to 1 cell ($1/10^8=10^{-8}$, by 8 decades of cell killing) is:
$8 \times 6.9 = 55.2$ Gy

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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 $2 \times 2 \times 2 = 8$
- 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.

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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

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