Chapter 3 – Lecture 1

Cell Survival Curves

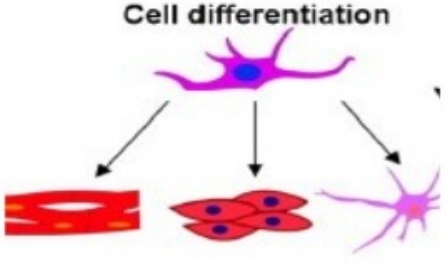
9/16/2024

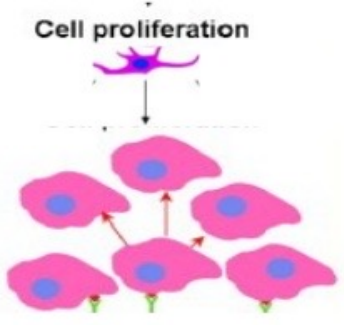
Lecture Outline

- **Reproductive Integrity**
- Mechanisms of Cell Killing
- The *In Vitro* Survival Curve
- The Shape of the Survival Curve
- Appendix – Bystander Effect
- Review Questions

Definition of Cell Death

- Cell death may mean different things in different context





- For **differentiated cells** that do not divide (e.g., nerve, muscle), death can be defined as the loss of a specific function
- **For proliferating cells (e.g., bone marrow stem cells, intestinal epithelium, tumor), death can be defined as loss of the capacity for sustained proliferation, i.e., loss of *reproductive integrity***

Reproductive Death

- Following irradiation, cells may still be intact and able to produce proteins, synthesize new DNA and even go through one or two cell divisions, but if it has **lost the capability to reproduce indefinitely**, it is considered dead

- This is also known as ***reproductive death***

Mean Lethal Dose

- Very high radiation doses (100 Gy) is necessary to cause the breakdown of all cellular functions in nonproliferating systems
- In contrast, the **mean lethal dose** for loss of reproductive capability is usually less than **2 Gy**

Cell Survival and Survival Curve

- Under this definition, a **survivor** means it has retained its reproductive integrity and is able to proliferate indefinitely to produce a large clone or colony, and is said to be ***clonogenic***

Relevance to Radiotherapy

For a tumor to be eradicated, it is only necessary that cells be “killed” in the sense that they are rendered unable to divide and cause further growth and spread of the malignancy

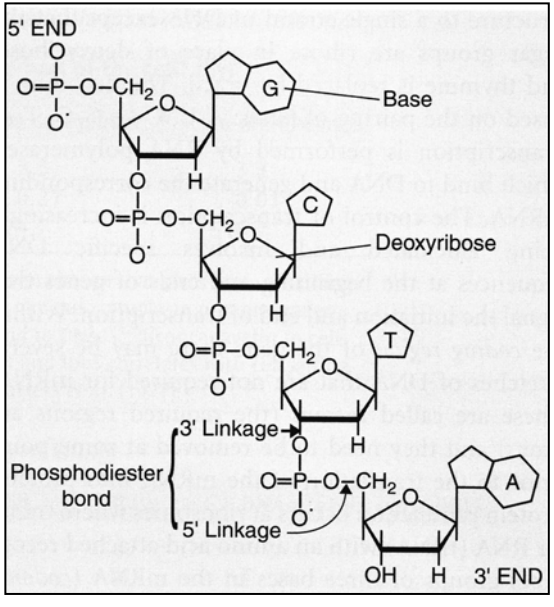
- A **survival curve** describes the relationship b/w the *radiation dose* and the *proportion of cells that survive*

Lecture Outline

- Reproductive Integrity
- **Mechanisms of Cell Killing**
- The *In Vitro* Survival Curve
- The Shape of the Survival Curve
- Appendix – Bystander Effect
- Review Questions

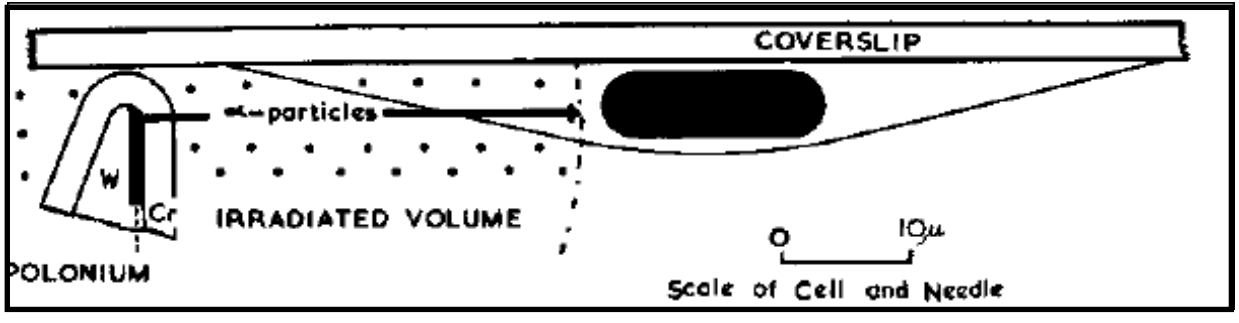
Critical Target of Ionizing Radiation

- The biologic effects of radiation result principally from damage to **DNA**
- Main problem = strand breaks
- Non-rejoined breaks → cell death
- Incorrectly rejoined breaks → mutations
- Damage of bases → mutations



Nucleus vs. Cytoplasm

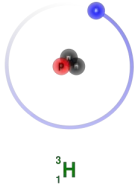
- Microbeam experiments with a particle from polonium show that the cell nucleus is the sensitive site as opposed to the cytoplasm



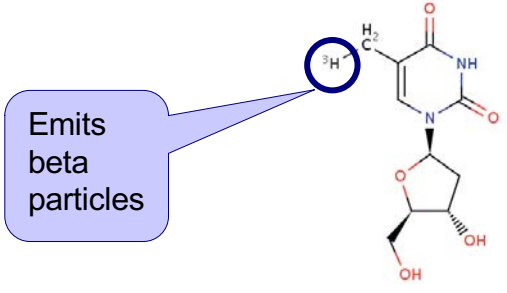
Microbeam experiment

Chromosomal DNA as the Principal Target

- Cells are killed by radioactive *tritiated thymidine* incorporated during synthesis into the cell



Tritium



- *Halogenated pyrimidines* incorporated into DNA in place of thymidine increase radiosensitivity
  - Substituted deoxyuridines, which are not incorporated into DNA have no such effect

Chromosomal DNA as the Principal Target

- Factors that modify cell lethality such as type of radiation, oxygen status, and dose rate also affect chromosome damage in a fashion qualitatively and quantitatively similar
- Radiosensitivity correlates well with the chromosome volume

Critical Target of Ionizing Radiation

- The biologic effects of radiation result principally from damage to **DNA**

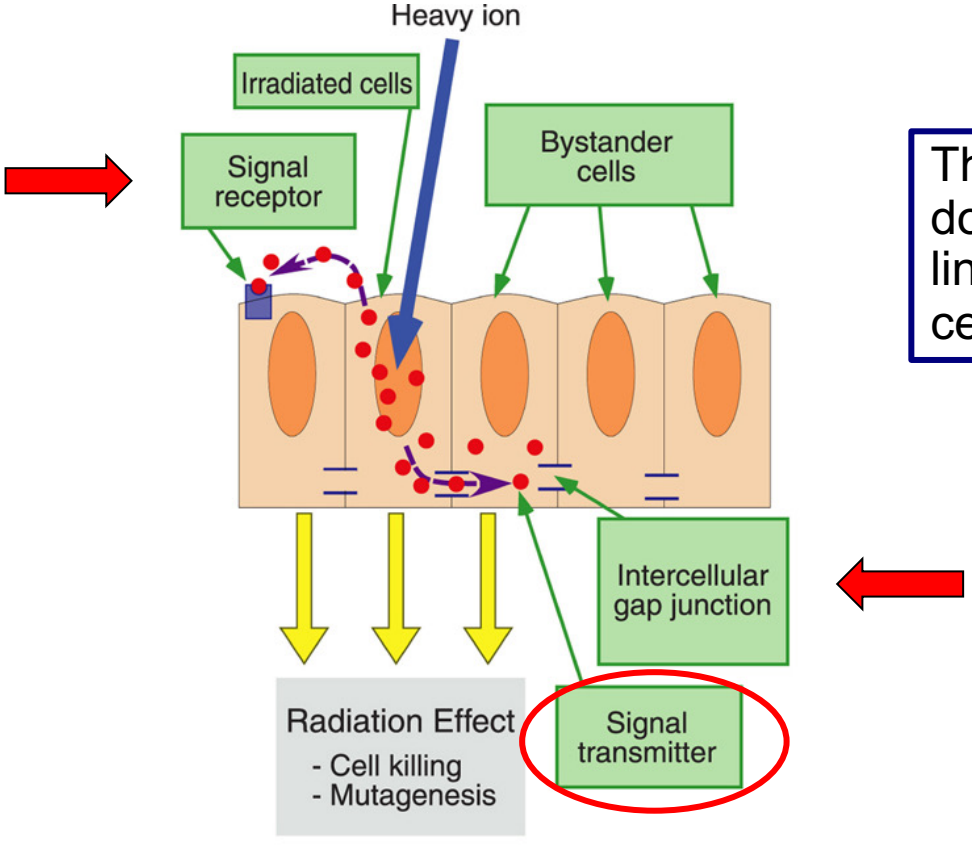
However, this is NOT the whole story!

The Bystander Effect

- In addition to direct damage to DNA, a ***bystander effect*** has also been implicated in various radiation-induced biological effects, including chromosomal aberrations and cell killing

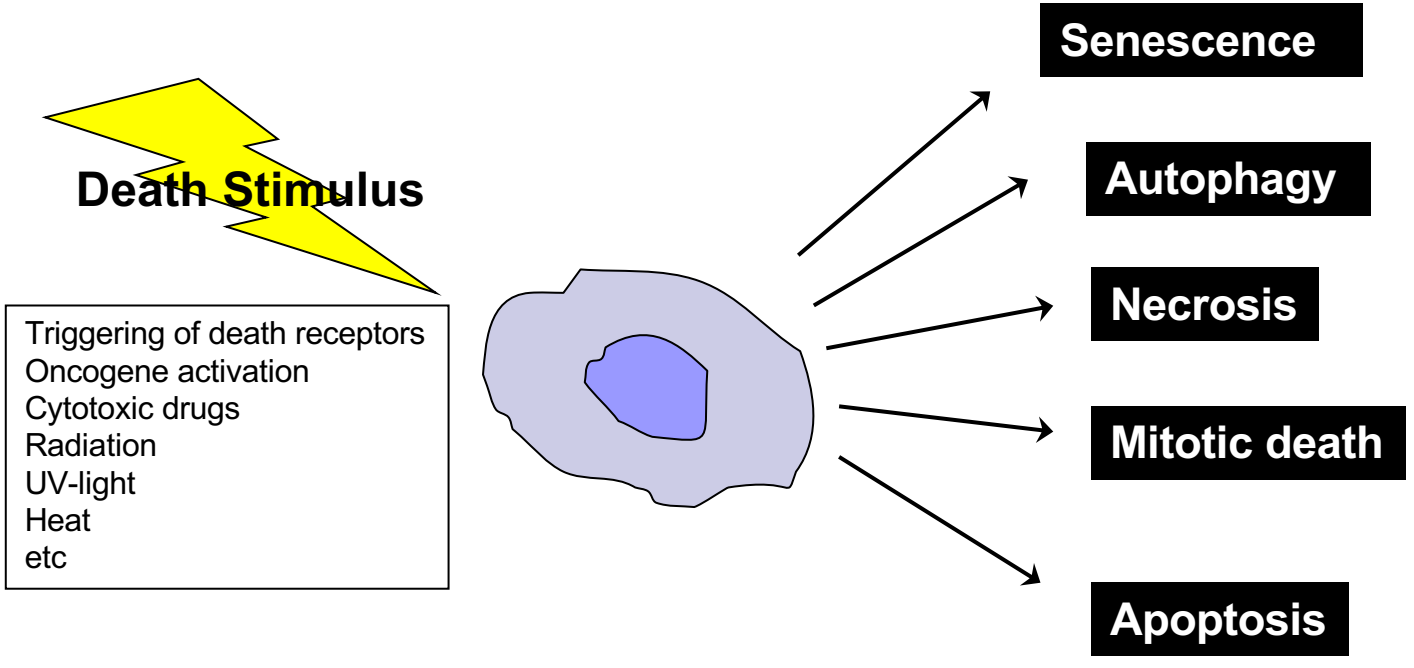
Bystander Effect = the induction of biologic effects in cells that are ***not directly*** traversed by a charged particle, but are ***in close proximity*** to the cells that are

The Bystander Effect



The bystander effect has been documented in both cancer cell lines and normal, untransformed cells

Modes of Cell Death



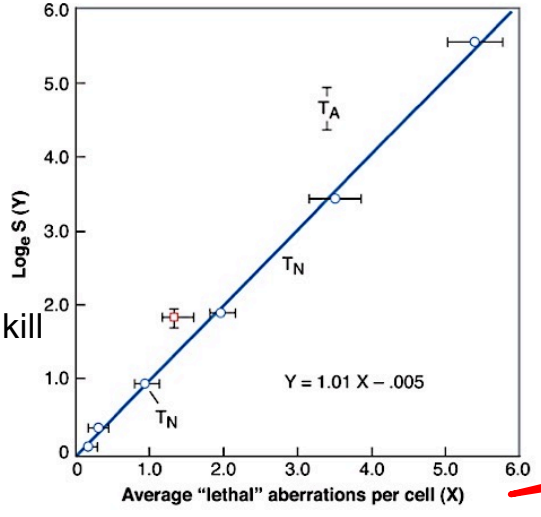
Outcome = loss of reproductive integrity

Mitotic Death

- Most common form of cell death after radiation
- Cells die attempting to divide because of damaged chromosomes
- Death may occur in the first or subsequent division following radiation
- Results from **asymmetric exchange-type aberrations** (e.g., rings, dicentrics)

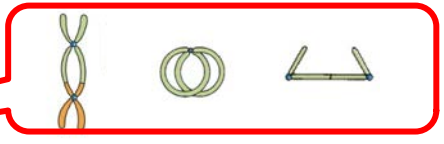
Chromosomal Aberrations & Survival – Experimental Data

Human fibroblasts exposed to X-ray



Log of kill

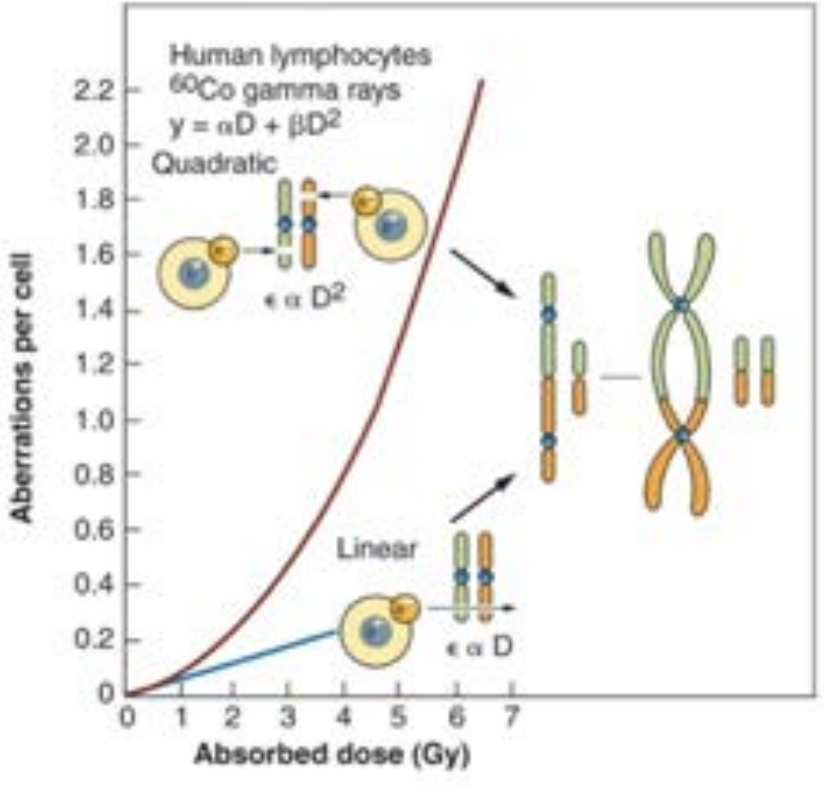
There is virtually a one-to-one correlation b/w $\log_e S$ and the average number of lethal aberrations per cell



Asymmetric exchange-type aberrations represent the principal mechanism for radiation-induced mitotic death

Cornforth & Bedford 1987

Dose Response – “2 Hits”



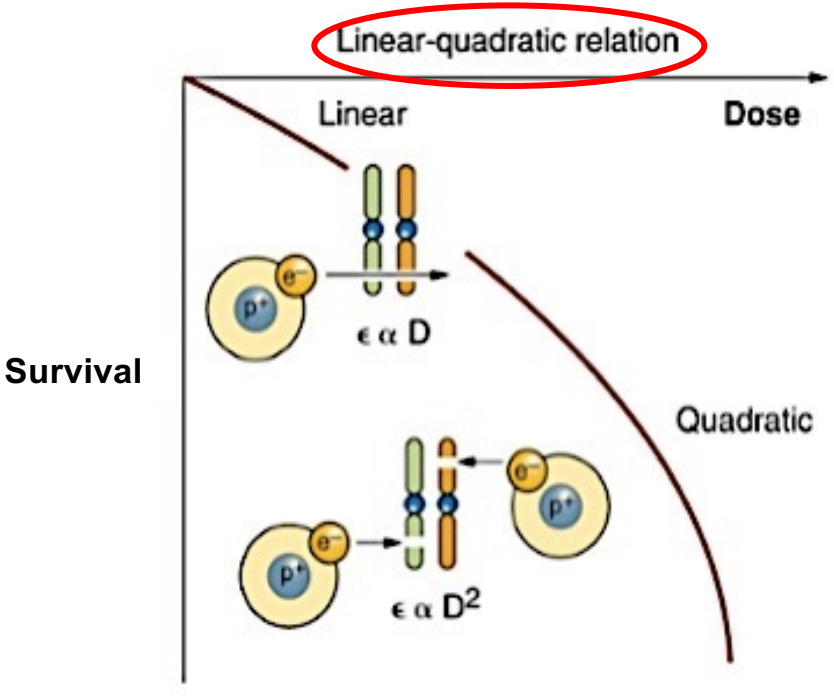
At Higher Doses

The two breaks are more likely to be caused by separate electrons → the probability of an exchange aberration is proportional to square of dose

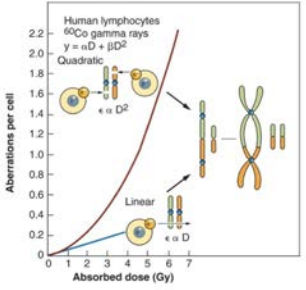
At Low Doses

Both breaks may be produced by the same electron → the probability of an exchange aberration is proportional to dose

Chromosomal Aberrations & Survival – Schematic



In cells die by mitotic death, survival follows a linear-quadratic function of the dose



At low doses, both breaks may be produced by the same electron → the probability of an exchange aberration is **proportional to dose** → decrease of **survival** is **proportional to the dose**

At higher doses, the two breaks are more likely to be caused by separate electrons → the probability of an exchange aberration is **proportional to square of dose** → decrease of **survival** is **proportional to the square of dose**

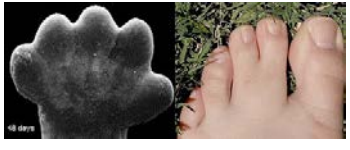
Apoptosis

Apoptosis (ay-paw-TOE-sis): A Natural End to Life

Apoptosis is a Greek word that translates to "falling off" or "falling away." It's often used to describe the natural world: autumn leaves as they tumble from the branch; flower petals wilting and drifting to earth. In Greek, the second "p" is silent, and so the traditional and most correct pronunciation is "ay-paw-TOE-sis." More recently, some people have pronounced it as an English word: "ay-POP-toe-sis."

Apoptosis

- It is a cellular response to "insult" such as UV light, chemical or physical damage, or a viral infection
- This “insult” starts **a cascade of events** which lead to the destruction of the cell
- Apoptosis is common in embryonic development in which some tissues become obsolete

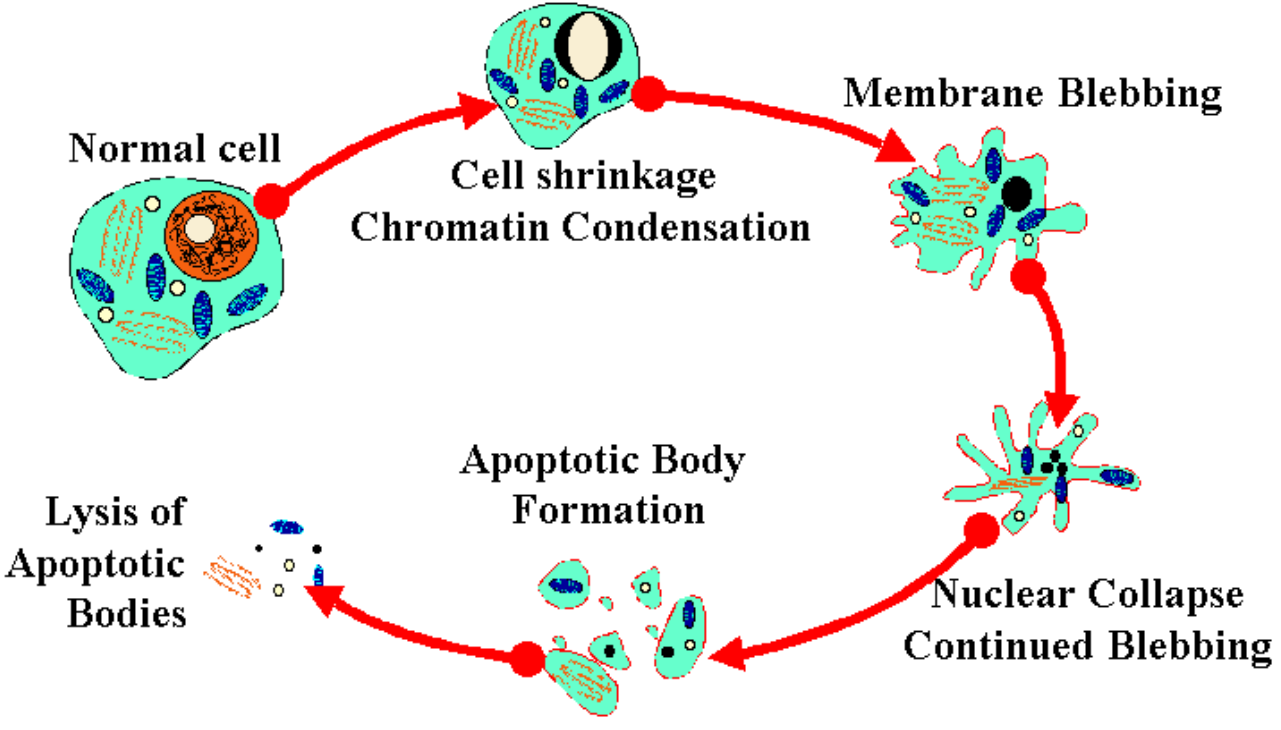


- This mechanism is often called ***"programmed cell death"*** as it is an innate response of the cell which protects the rest of the organism from a potentially harmful agent

Apoptosis

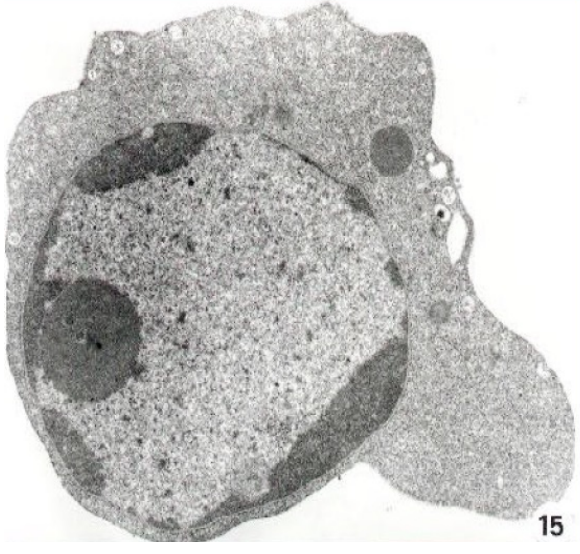
Morphologic Hallmark = condensation of the nuclear chromatin

Biochemical Hallmark = DNA fragmentation

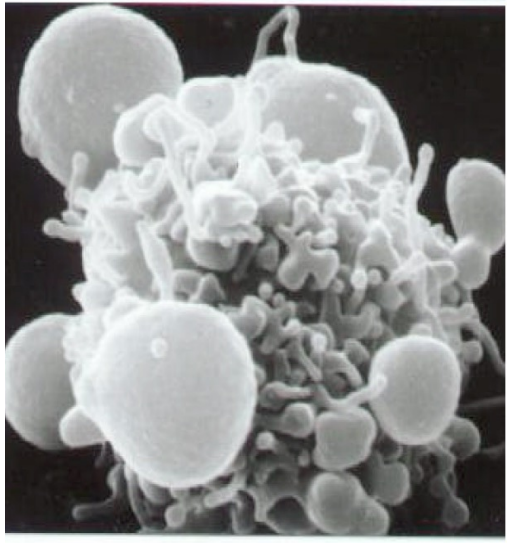


Morphologic Features of Apoptosis

Electron Microscopy

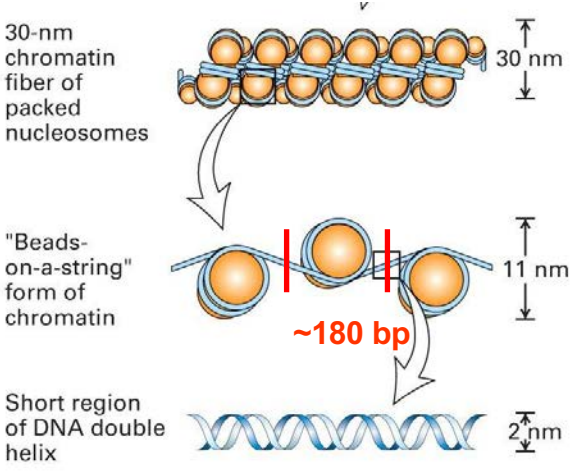


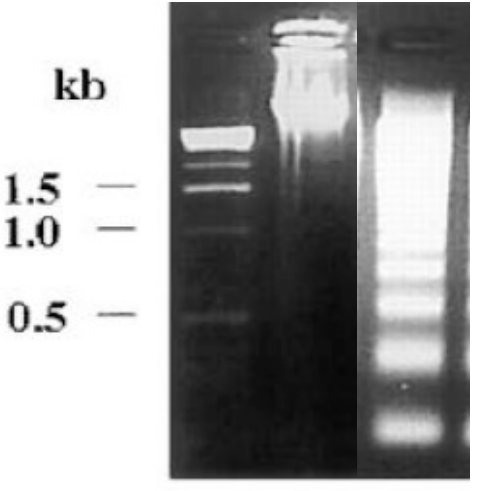
Chromatin Condensation



Membrane Blebbing

Biochemical Hallmark of Apoptosis





DNA laddering

DNA fragmentation occurs in the linker region b/w nucleosomes

DNA fragments are multiples of 185 base pairs

Apoptotic Death

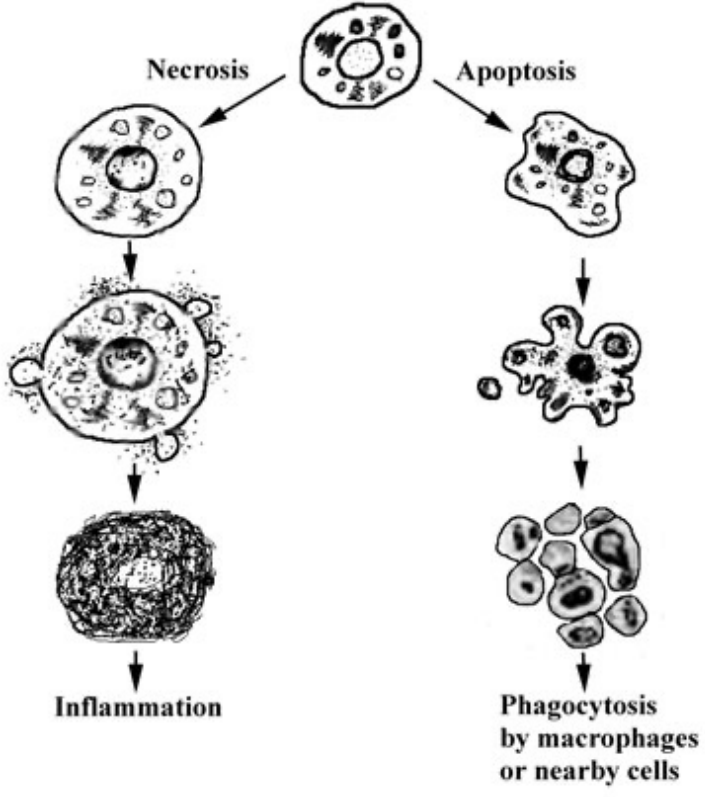
- As a mode of radiation-induced death, apoptosis is highly cell-type dependent
- Hemopoietic and lymphoid cells are particularly prone to rapid radiation-induced apoptosis
- Apoptosis after radiation is commonly ***p53***-dependent; ***bcl-2*** is a suppressor of apoptosis

Apoptosis vs. Necrosis

Breakdown of cellular membrane

Leakage of intracellular proteins

Inflammation

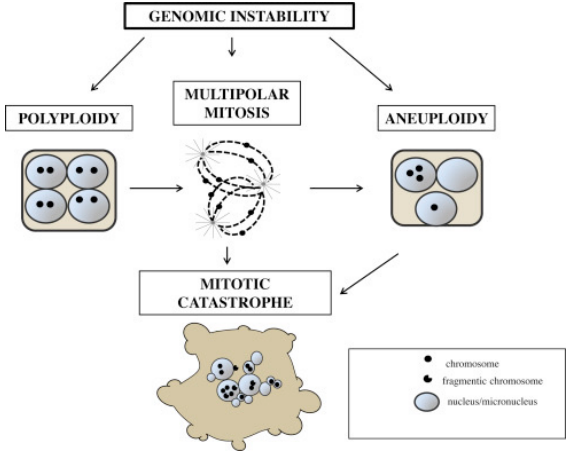


Apoptosis vs. Necrosis

Apoptosis	Necrosis
• Chromatin condensation	• Nuclear swelling
• Cell Shrinkage	• Cell Swelling
• Preservation of Organelles and cell membranes	• Disruption of Organelles
• Rapid engulfment by neighboring cells preventing inflammation	• Rupture of cell and release of cellular contents
• Biochemical Hallmark: DNA FRAGMENTATION	• Inflammatory response

Mitotic Catastrophe

- Mitotic catastrophe results from aberrant mitosis and can produce giant, multinucleated aneuploid cells that remain metabolically active
- Mitotic catastrophe is associated with deficiencies of the G2 and mitotic spindle checkpoints
- Often such cells will fail in the final stage of karyokinesis (nuclear cleavage) and cytokinesis (cellular cleavage) which results in giant cells reforming a single nuclear envelope with tetraploid DNA content and double the normal G1 chromosome number
- Cells undergoing mitotic catastrophe may subsequently die by apoptosis and mitotic cell death, suggesting that mitotic catastrophe may not be a specific cell death program but precedes other modes of cell death

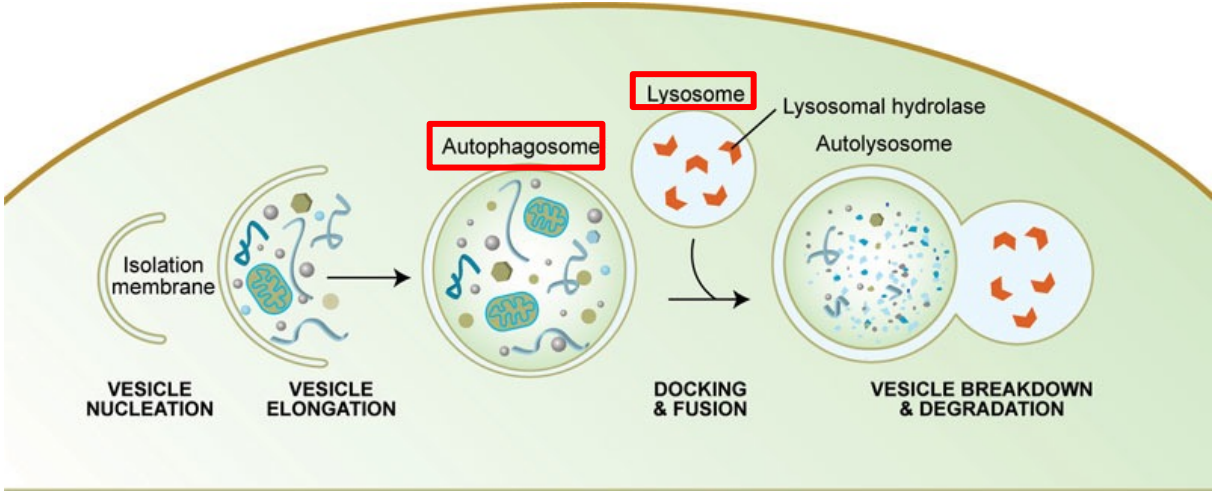


Autophagy

III. **Cellular responses to radiation**
a. Mechanisms of cell death
i. Mechanisms and major characteristics of pathways of radiation-induced apoptosis, necrosis, autophagy, and senescence

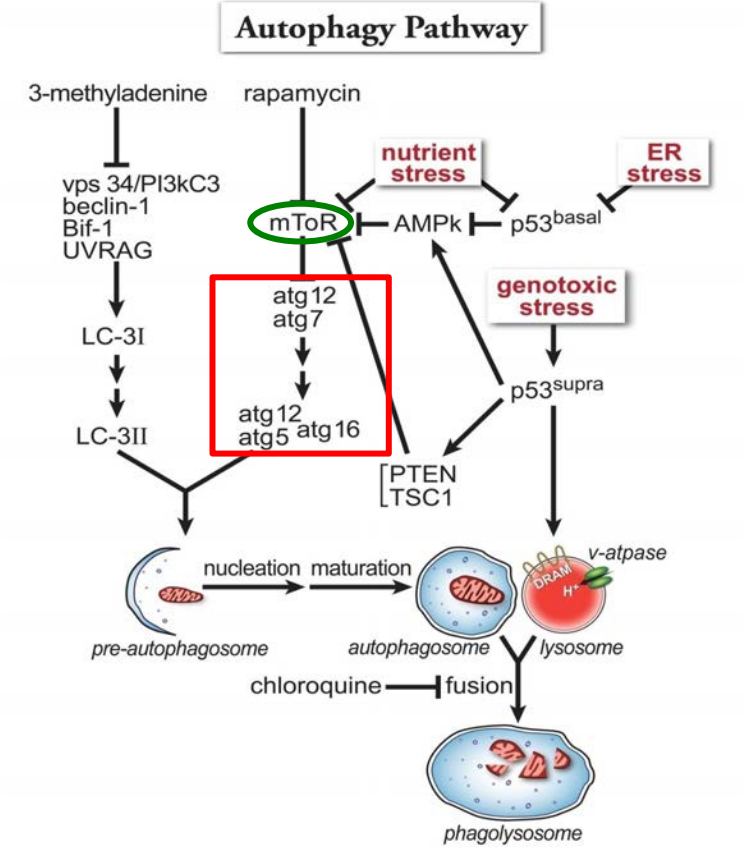
- Derived from the the Greek “to eat” (“phagy”) “oneself” (“auto”)
- Autophagy is the process by which cells recycle their own non-essential, redundant, or damaged organelles and marocromolecular components
- Autophagic death is also termed type II programmed death

Autophagy



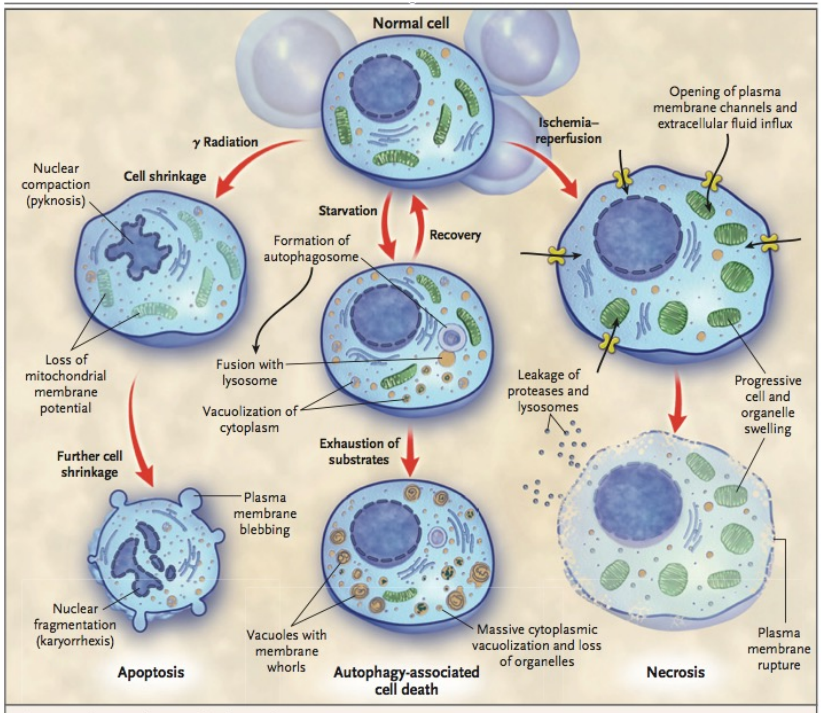
- The hallmark of autophagy is **autophagosome**, which can engulf bulk cytoplasm nonspecifically, including entire organelles, or target cargos specially
- Autophagosomes fuse with **lysosomes**, where acid hydrolases catabolize the ingested material into metabolic substrates

Autophagy Pathway



- A complex set of autophagy-related genes regulates the formation of autophagosomes
- Among these are ***Atg***s, which is required for the elongation of the autophgosomal membrane
- Additional regulators include **mTOR**, a serine-threonine protein kinase that integrates input from cellular nutrients, growth factors and cellular redox state to inhibit autoagosome formation

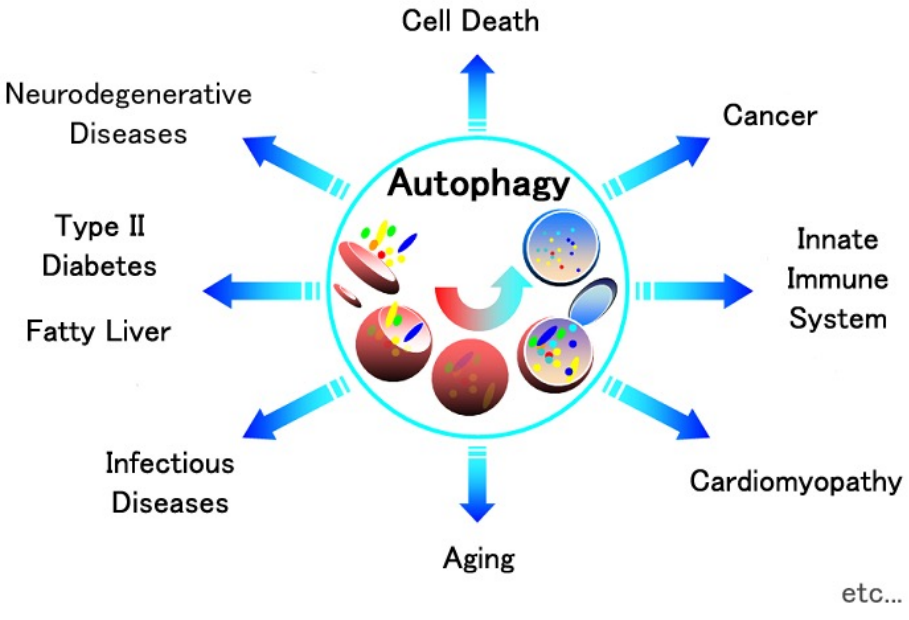
Autophagy



- Autophagy allows a starving cell, or a cell that is deprived of growth factors to survive = protective & adaptive
- However, cells that do not receive nutrients for extended periods ultimately digest all available substrates and die = autophagic death, or Type II programmed death
- Supplying nutrients before this critical point would restore the cell's health

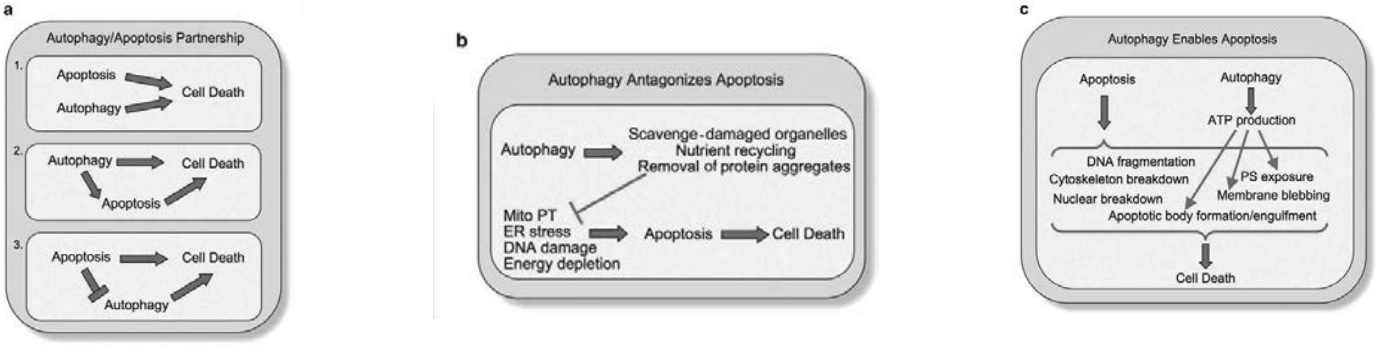
Autophagy – Clinical Implication

Another essential function is to remove potentially harmful proteins to protect the cells against diseases and infection by pathogens



Autophagy & Cancer

- Chemotherapeutic agents and radiotherapy have also been reported to induce autophagy and autophagic cell death



In some cellular settings, it can serve as a **cell survival pathway**, suppressing apoptosis, and in others, it **can lead to death itself**, either in collaboration with apoptosis or as a back-up mechanism when the former is defective.

A potential new target for anticancer therapy!

2016 Nobel Prize

Press Release

2016-10-03

The Nobel Assembly at Karolinska Institutet has today decided to award

the 2016 Nobel Prize in Physiology or Medicine

to

Yoshinori Ohsumi

for his discoveries of mechanisms for autophagy

2016 Nobel Prize

Summary

This year's Nobel Laureate discovered and elucidated mechanisms underlying *autophagy*, a fundamental process for degrading and recycling cellular components.

The word *autophagy* originates from the Greek words *auto-*, meaning "self", and *phagein*, meaning "to eat". Thus,autophagy denotes "self eating". This concept emerged during the 1960's, when researchers first observed that the cell could destroy its own contents by enclosing it in membranes, forming sack-like vesicles that were transported to a recycling compartment, called the *lysosome*, for degradation. Difficulties in studying the phenomenon meant that little was known until, in a series of brilliant experiments in the early 1990's, Yoshinori Ohsumi used baker's yeast to identify genes essential for autophagy. He then went on to elucidate the underlying mechanisms for autophagy in yeast and showed that similar sophisticated machinery is used in our cells.

Ohsumi's discoveries led to a new paradigm in our understanding of how the cell recycles its content. His discoveries opened the path to understanding the fundamental importance of autophagy in many physiological processes, such as in the adaptation to starvation or response to infection. Mutations in autophagy genes can cause disease, and the autophagic process is involved in several conditions including cancer and neurological disease.

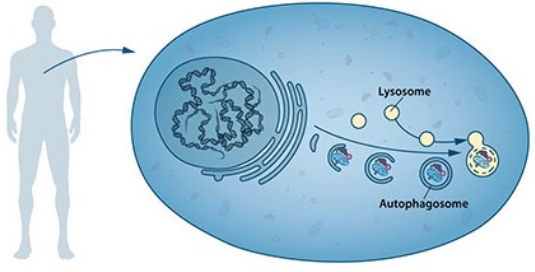


Figure 1: Our cells have different specialized compartments. Lysosomes constitute one such compartment and contain enzymes for digestion of cellular contents. A new type of vesicle called autophagosome was observed within the cell. As the autophagosome forms, it engulfs cellular contents, such as damaged proteins and organelles. Finally, it fuses with the lysosome, where the contents are degraded into smaller constituents. This process provides the cell with nutrients and building blocks for renewal.

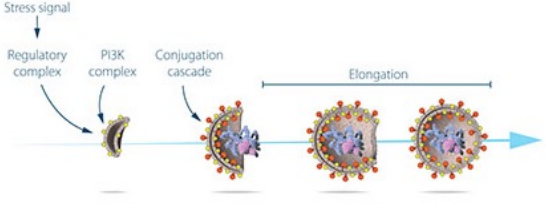
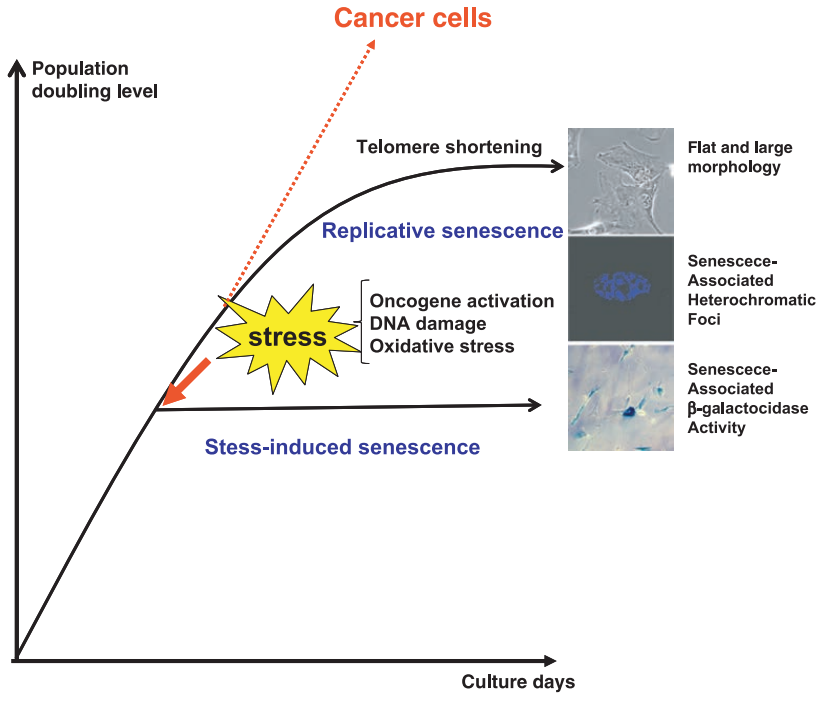


Figure 3: Ohsumi studied the function of the proteins encoded by key autophagy genes. He delineated how stress signals initiate autophagy and the mechanism by which proteins and protein complexes promote distinct stages of autophagosome formation.

Senescence

= **Irreversible** Growth Arrest



Replicative Senescence

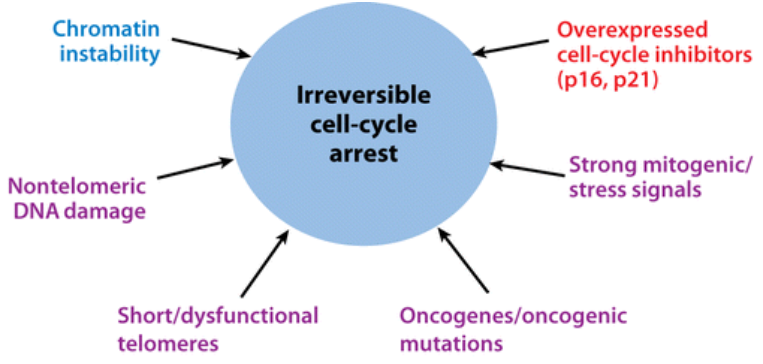
Normal human fibroblasts enter senescence after a finite # of cell divisions *in vitro* caused by telomere shortening

Stress-Induced Senescence

Cellular senescence can also be induced prematurely by a number of cellular stresses such as oncogenic stimuli, oxidative stress and DNA damage before reaching their limits of replicative life span

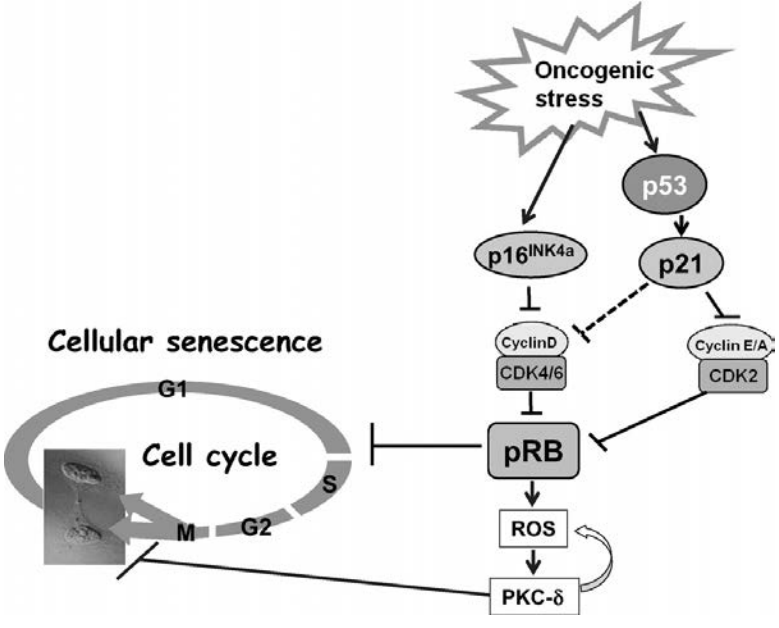
Senescence

- Cellular senescence is a unique response to the accumulation of damage to a cell



- It is thus a **tumor suppressor mechanism**
- Though reproductively inhibited, cells can still be metabolic active

Senescence – Mechanism



- Oncogenic stress induces **p16INK4a** and p53-target **p21**
- When **RB** is fully activated by high-level expression of p16INK4a, mitogenic signals in turn, ↑ the level of reactive oxygen species (**ROS**) and elicit a positive feedback activation of the ROS-PKC-δ signaling pathway
- Elevated levels of p16INK4a therefore establish the autonomous activation of the pathway, leading to an **irrevocable block** to cytokinesis in human senescent cells

Characteristics of Different Types of Cell Death

Type of cell death	Morphological changes			Biochemical features	Common detection methods
	Nucleus	*Cell membrane*	*Cytoplasm*		
Apoptosis	Chromatin condensation; nuclear fragmentation; DNA laddering	Blebbing	Fragmentation (formation of apoptotic bodies)	Caspase-dependent	Electron microscopy; TUNEL staining; annexin staining; caspase-activity assays; DNA-fragmentation assays; detection of increased number of cells in subG1/G0; detection of changes in mitochondrial membrane potential
Autophagy	Partial chromatin condensation; no DNA laddering	Blebbing	Increased number of autophagic vesicles	Caspase-independent; increased lysosomal activity	Electron microscopy; protein-degradation assays; assays for marker-protein translocation to autophagic membranes; MDC staining
Mitotic catastrophe	Multiple micronuclei; nuclear fragmentation	–	–	Caspase-independent (at early stage) abnormal CDK1/cyclin B activation	Electron microscopy; assays for mitotic markers (MPM2); TUNEL staining
Necrosis	Clumping and random degradation of nuclear DNA	Swelling; rupture	Increased vacuolation; organelle degeneration; mitochondrial swelling	–	Electron microscopy; nuclear staining (usually negative); detection of inflammation and damage in surrounding tissues
Senescence	Distinct heterochromatic structure (senescence-associated heterochromatic foci)	–	Flattening and increased granularity	SA-β-gal activity	Electron microscopy; SA-β-gal staining; growth-arrest assays; assays for increased p53, INK4A and ARF levels (usually increased); assays for RB phosphorylation (usually hypophosphorylated); assays for metalloproteinase activity (usually upregulated)

CDK1, cycline-dependent kinase 1; MDC, monodansylcadaverine; MPM2, mitotic phosphoprotein 2; SA-β-gal, senescence-associated β-galactosidase; RB, retinoblastoma protein.

Lecture Outline

- Reproductive Integrity
- Mechanisms of Cell Killing
- **The *In Vitro* Survival Curve**
- The Shape of the Survival Curve
- Appendix – Bystander Effect
- Review Questions

In Vitro Techniques

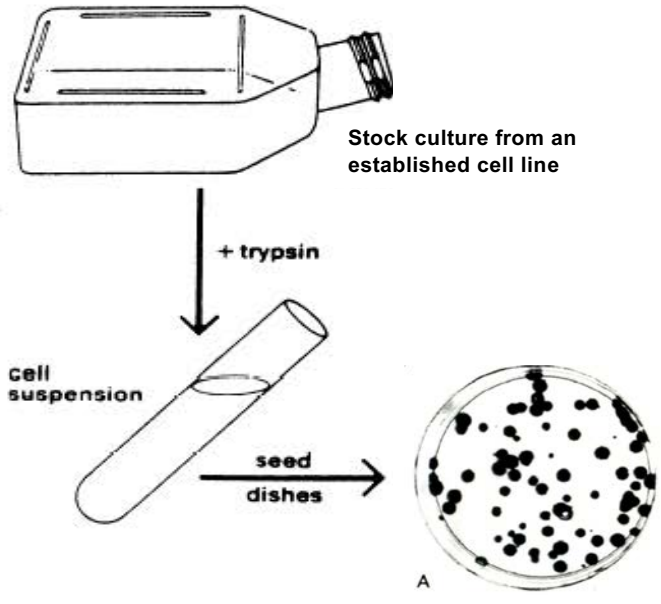
In vitro – performed or taking place in a test tube, culture dish, or elsewhere outside a living organism as opposed to ***in vivo***.

- With modern techniques of tissue culture, cells from tumors and many normal regenerative tissues can grow and form colonies ***in vitro***

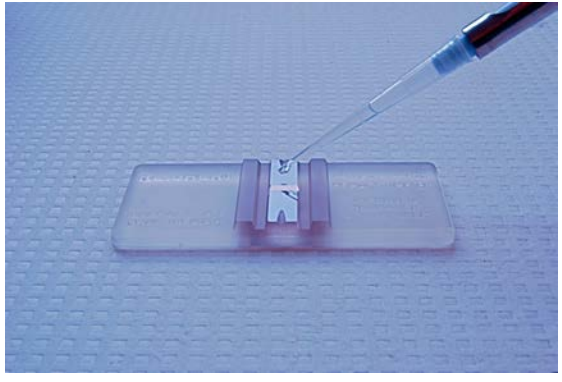
- However, fresh explants often grow well for a few weeks before they peter out and die
- A few pass through a “crisis” and become immortal; these are the **established cell lines**
- Established cell lines have been used extensively to study survival curves

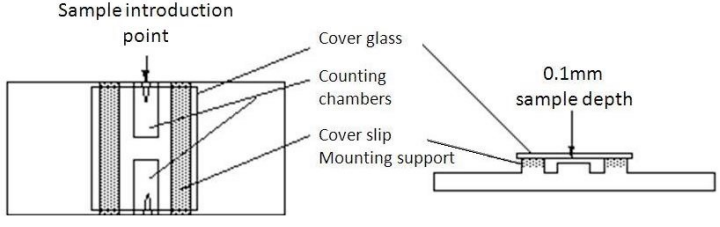
Clonogenic Survival Assay

- Cells from an actively growing stock are harvested by gentle scraping or by the use of trypsin
- The number of cells per unit volume is determined
- Known numbers of cells are plated into fresh dishes
- If allowed to incubate for 1-2 weeks, clonogenic cells will form macroscopically visible colonies that can be fixed, stained and counted

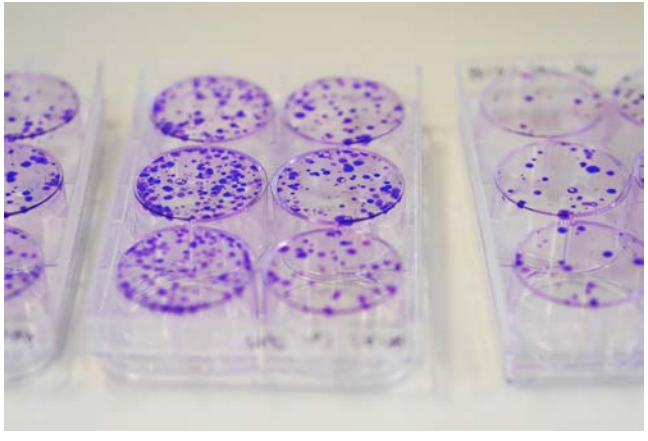


Clonogenic Survival Assay





Hemocytometer Counting of Cells



Crystal Violet Cell Colony Staining

Plating Efficiency

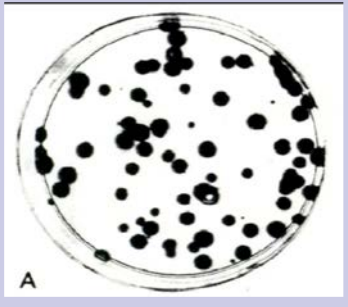
- If 100 cells are seeded into the dish, it is seldom to obtain 100 colonies
- This is caused by a variety of reasons such as
  - Suboptimal growth medium
  - Errors and uncertainties in counting the cell suspension
  - Trauma of trypsinization and handling
- **Plating efficiency** indicates the % of cells seeded that grow into colonies

$$\text{Plating Efficiency} = \frac{\text{\# observed colonies}}{\text{\# of plated cells}}$$

Unirradiated with 100 cells seeded

70 colonies counted

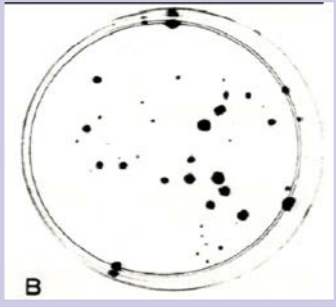
PE = 70/100 = 70%



Surviving Fraction

- In a parallel dish, **2,000** cells are seeded and exposed to **8 Gy** of X-rays, then incubated
- Some cells remain single and have not divided
- Some cells manage to complete one or two divisions
- Some cells grow into large colonies; **these cells have retained reproductive integrity, and are said to have survived**

2000 cells seeded and then irradiated

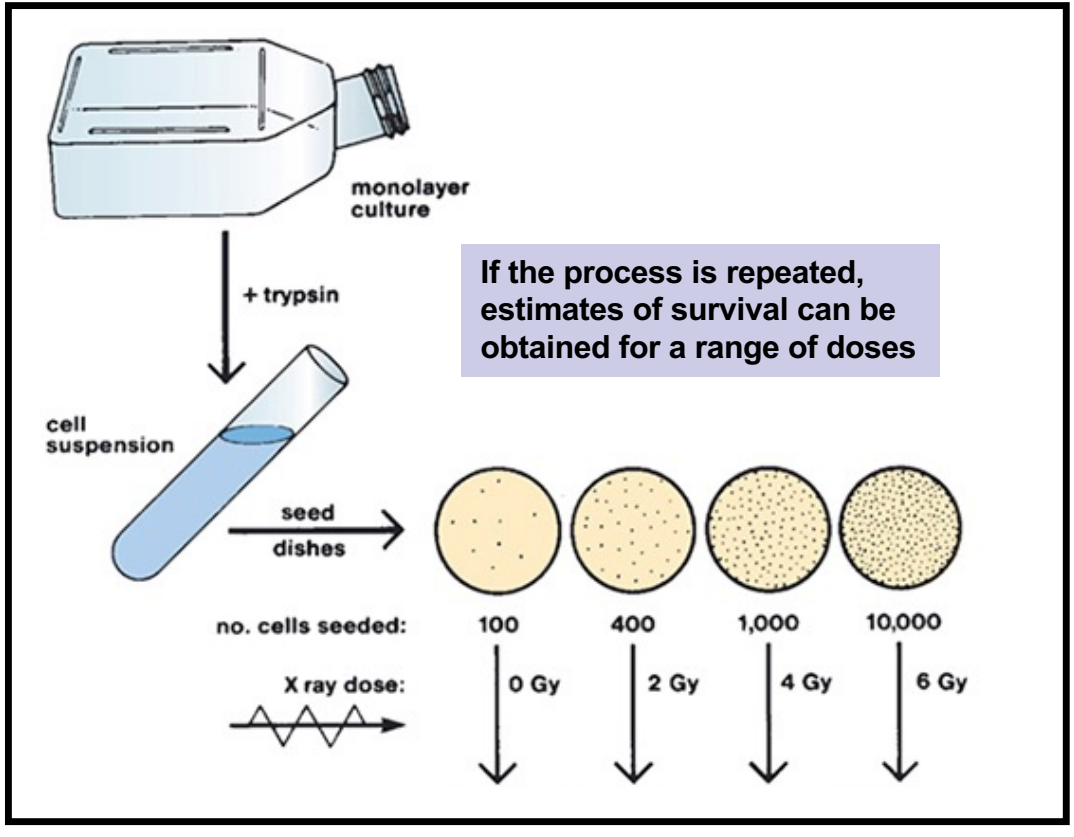


32 colonies counted

Surviving fraction = 32/(2000x0.7) = 0.023

$$\text{Surviving Fraction} = \frac{\text{Colonies counted}}{\text{Cells seeded x PE}}$$

Generating a Cell Survival Curves

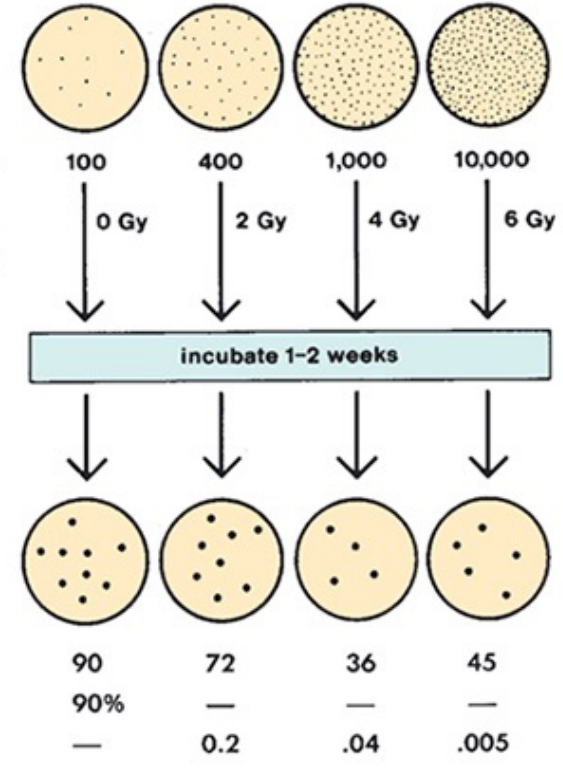


If the process is repeated, estimates of survival can be obtained for a range of doses

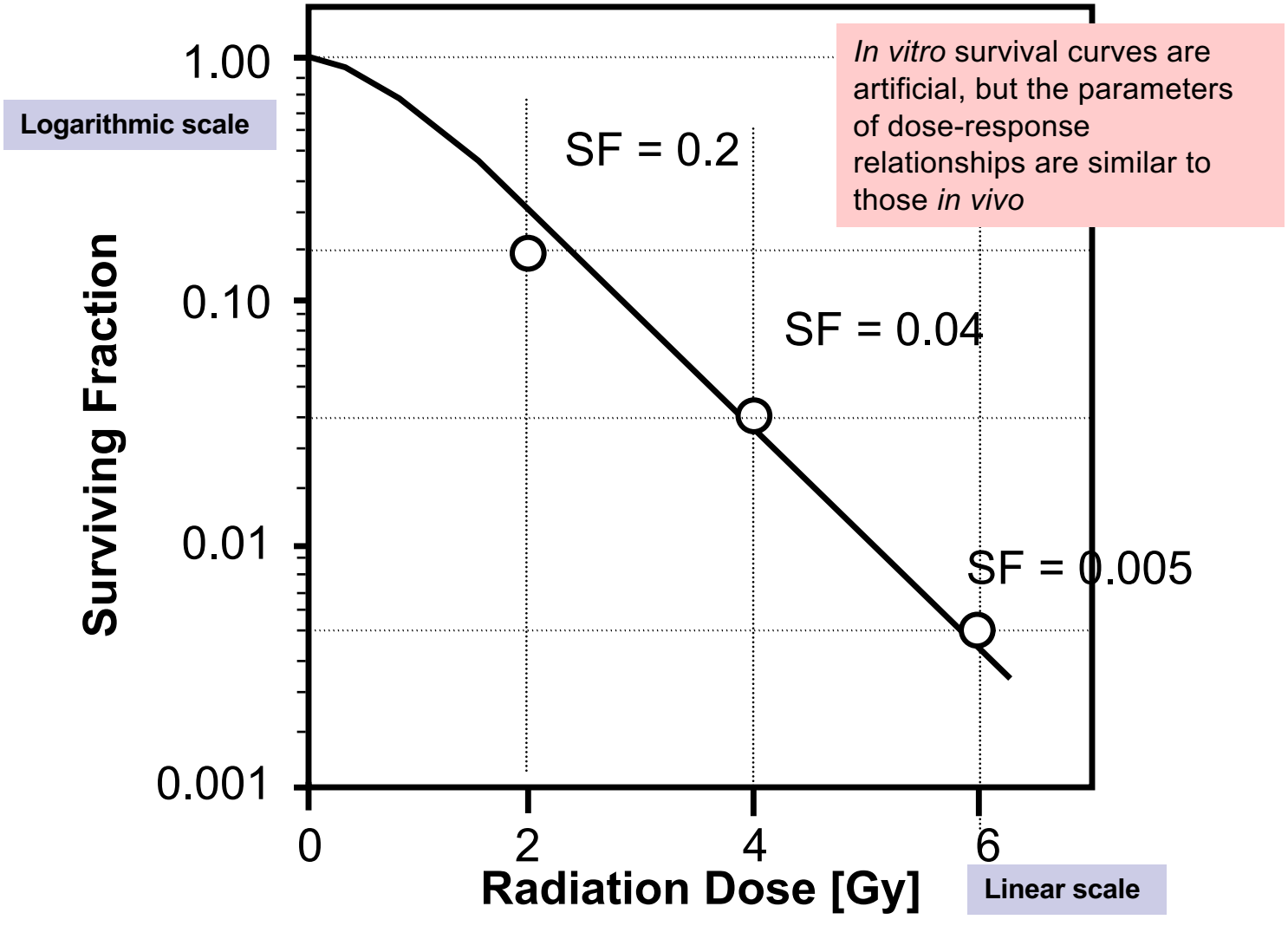
⇦

The # of cells seeded per dish is adjusted according to the dose

Generating a Cell Survival Curve



colonies counted	→	90	72	36	45
plating efficiency	→	90%	—	—	—
surviving fraction	→	—	0.2	.04	.005

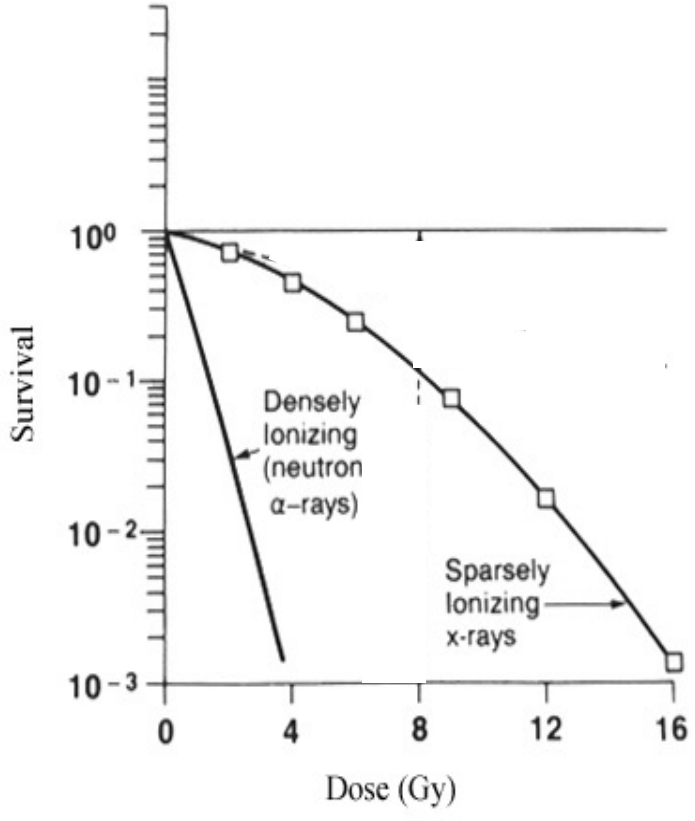

In vitro survival curves are artificial, but the parameters of dose-response relationships are similar to those *in vivo*
Logarithmic scale
1.00
0.10
0.01
0.001
Surviving Fraction
SF = 0.2
SF = 0.04
SF = 0.005
0
2
4
6
Radiation Dose [Gy]
Linear scale

Lecture Outline

- Reproductive Integrity
- Mechanisms of Cell Killing
- The *In Vitro* Survival Curve
- **The Shape of the Survival Curve**
- Appendix – Bystander Effect
- Review Questions

Cell Survival Curves

- Survival curves are plotted on a log-linear scale
- **High LET** – survival curves are linear (i.e., the SF is an exponential function of dose)
- **Low LET** – curve starts out straight → bends → straighten again



Qualitative Description

Shape of Cell Survival Curves

- The interpretation of the shape of the cell survival curve is still debated, as is the best way to fit these types of data mathematically

- 2 models will be discussed
  - **The Target Theory**
  - **The Linear-Quadratic Model**

Target Theory

- To inactivate a cell, each sensitive target needs to be “hit”
- **Simple Target Theory** – each cell has one target that needs to be hit only once for inactivation (also called **single target, single hit theory**)
- Target theory originated from work with **exponential dose response curves**

Linear Survival Curve

- Irradiation of cells with high-LET radiation produces linear survival curves
- The relationship between the surviving fraction S and the dose D is:

$$S = S_0 \, e^{-\alpha D}$$

S is the number of surviving cells
S_0 is the initial number of cells
α is the slope and a measure of the intrinsic radiation sensitivity
D is the radiation dose delivered

Linear Survival Curves

This relationship is more commonly represented as Surviving Fraction (SF) as a function of dose (D)

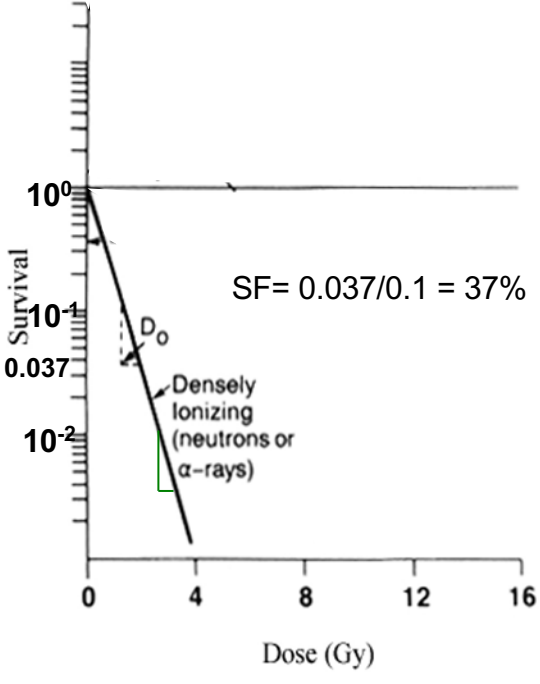
$$S = S_0 e^{-\alpha D}$$

$$SF = S/S_o = e^{-D/D_o}$$

(by defining D_0 as $1/\alpha$)

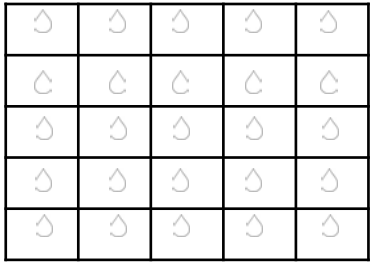
When $D = D_0$, $SF = e^{-1} = 0.37$

Therefore D_0 is the dose that ↓ SF to 37% of its initial value



D_0 is also the dose that delivers, *on average*, one lethal event per target = **mean lethal dose**

Raindrop Analogy

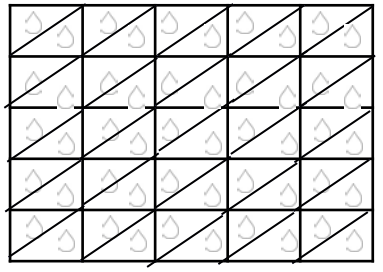


- If the number of raindrops is equal to the number of squares (100 squares, 100 raindrops), then all 100 squares would become wet with 100 raindrops, if the raindrops had fallen uniformly
- When raindrops are randomly directed towards the squares, 63% of the squares will be wet and 37% of the squares will be dry
- D_{37} or D_0 is the dose of radiation to reduce cell survival to 37% of the original cells

Simple Target Theory

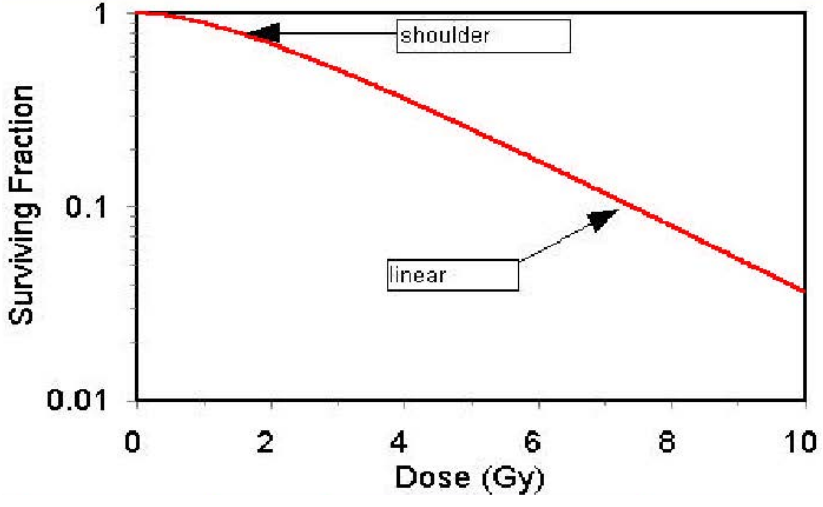
- Exponential dose response relationships are found in certain situations
  - Irradiation with high-LET radiation
  - Certain types of sensitive cells (e.g., hemopoietic stem cells)
  - Synchronized populations in M and G_2 (to be discussed in Chapter 4)

Single-Hit / Multi-Target Model



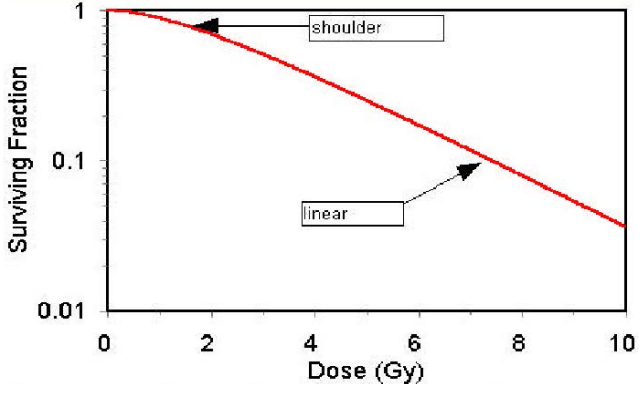
- Assume that each cell contains **n** targets, **each of which needs to be hit at least once** to inactivate the cell

$$SF = 1 - (1 - e^{-D/D_o})^n$$

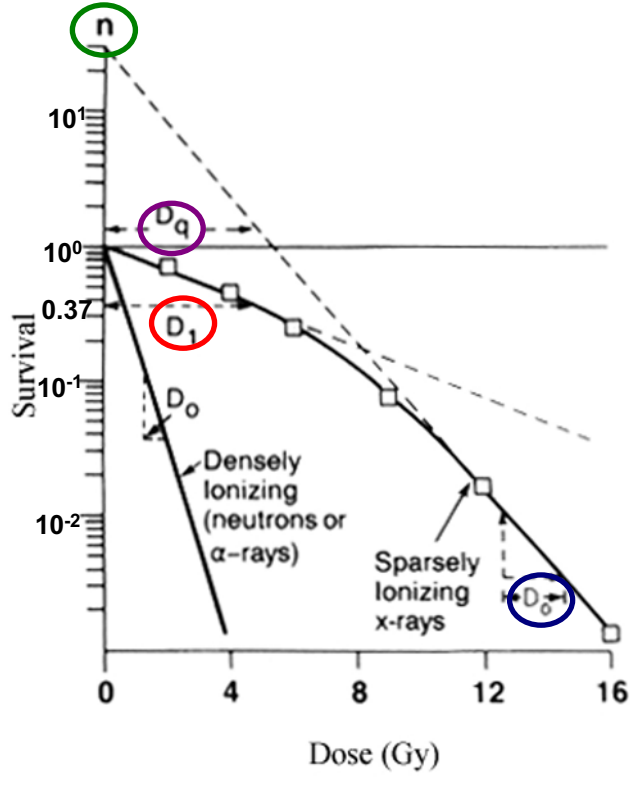


Single Hit/Multi-Target Model – Important Facts

- Survival curves for most mammalian cells exposed to **low-LET radiation** show some **curvature**
- The **initial low dose region** in which there is less cell inactivation per unit dose than at high doses is called the **shoulder**
- Often the **higher-dose region** tends towards a **straight line**



Target Model



α-ray or neutron – D_0 is adequate

X-ray or γ-ray
Characterized by 4 parameters

Initial slope (D_1) – Dose to ↓ SF to 37% of its previous value on initial portion of the curve

Final slope (D_0) – Dose to ↓ SF to 37% of its previous value on straight line portion of the curve

Extrapolation number (n) – Estimate of width of the shoulder

Quasi-threshold dose (D_q) – *Almost* a threshold dose, dose below which radiation purportedly has no effect

Parameters of Multi-target Model

D_0 = mean lethal dose
N (or n) = extrapolation number
D_q = quasi-threshold dose

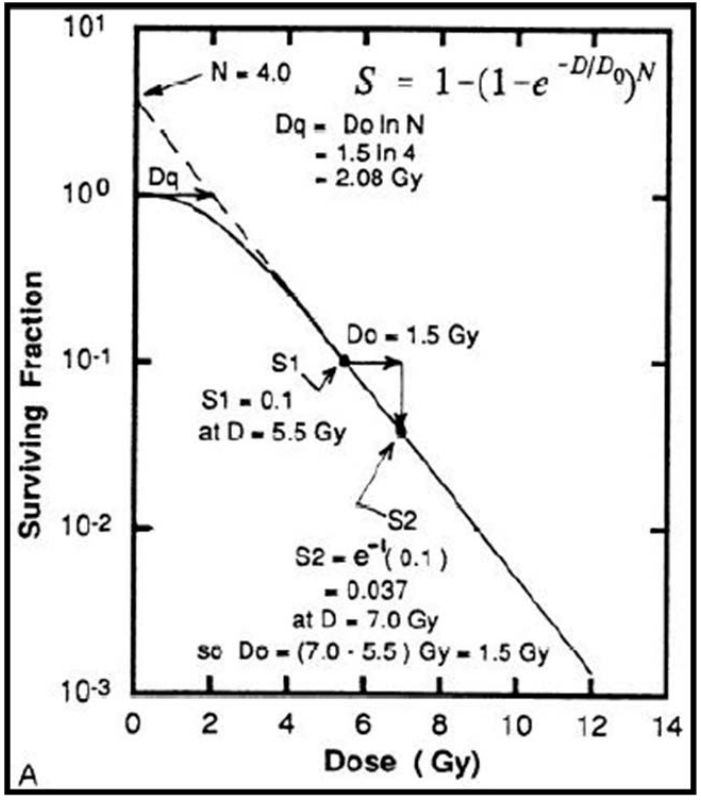
$$\frac{\ln N - \ln 1}{D_q} = \frac{\ln 0.1 - \ln 0.037}{D_0}$$

$$= \frac{\ln e}{D_0}$$

$$\therefore \frac{\ln N}{D_q} = \frac{1}{D_0}$$

$$i.e. \boxed{D_q = D_0 \ln N}$$

$\log_e N = D_q/D_0$



Target Model

- Major problem with this model is that there are too many parameters
- Need a mathematically simpler model with fewer “unknown” parameters
- The **Linear-Quadratic Model** meets these needs and has taken over as the model of choice

Lecture Outline

- Reproductive Integrity
- Mechanisms of Cell Killing
- The *In Vitro* Survival Curve
- The Shape of the Survival Curve
- **Appendix – Bystander Effect**
- Review Questions

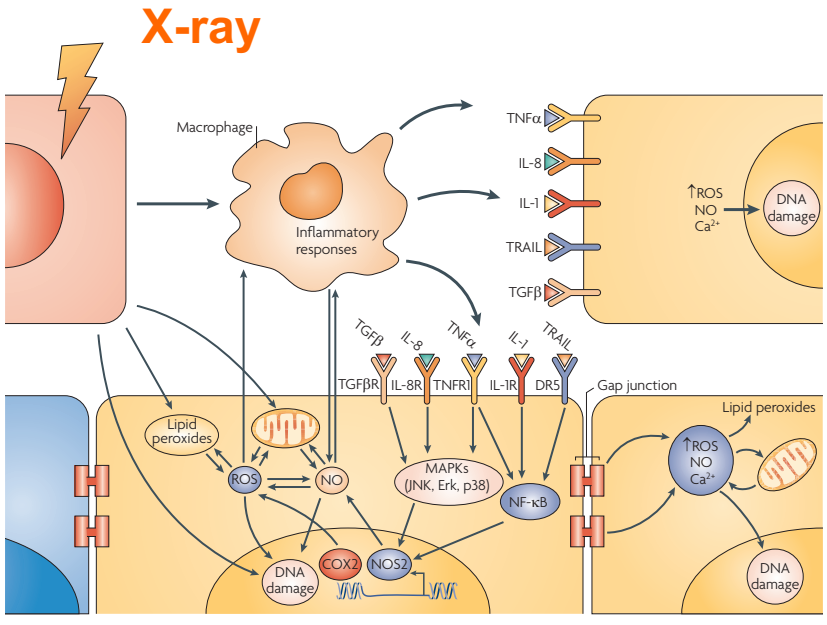
The Bystander Effect – Experiments

Single-particle microbeams – a known # of particles are delivered through the nucleus of specific cells, and the biologic effects in unirradiated close neighbors are studied

- e.g. When only 1% of cells were hit by α particle; 30% of the cells showed an ↑ in sister chromatid exchanges

Medium transfer – medium from irradiated cells are removed and added to unirradiated cells

Mechanisms



- Direct cell-cell-communication through **gap junctions**
- Release of **cytokine** signals into the extracellular matrix

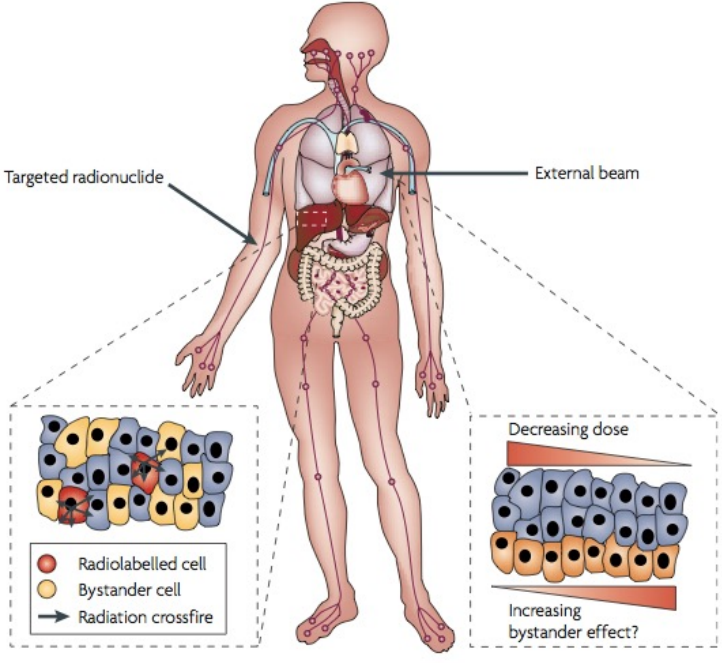
In vivo, **macrophages** may be important mediators, which in response to radiation-induced tissue damage release bystander signals

Some of the key pathways and mechanisms are now being elucidated, with roles for **cytokine-mediated signaling,** signal transduction through MAPKs and NF-κB along side the production of **reactive oxygen** and **nitrogen species**

Prise & O'Sullivan 2009; Nature Reviews 9: 351

Clinical Implications

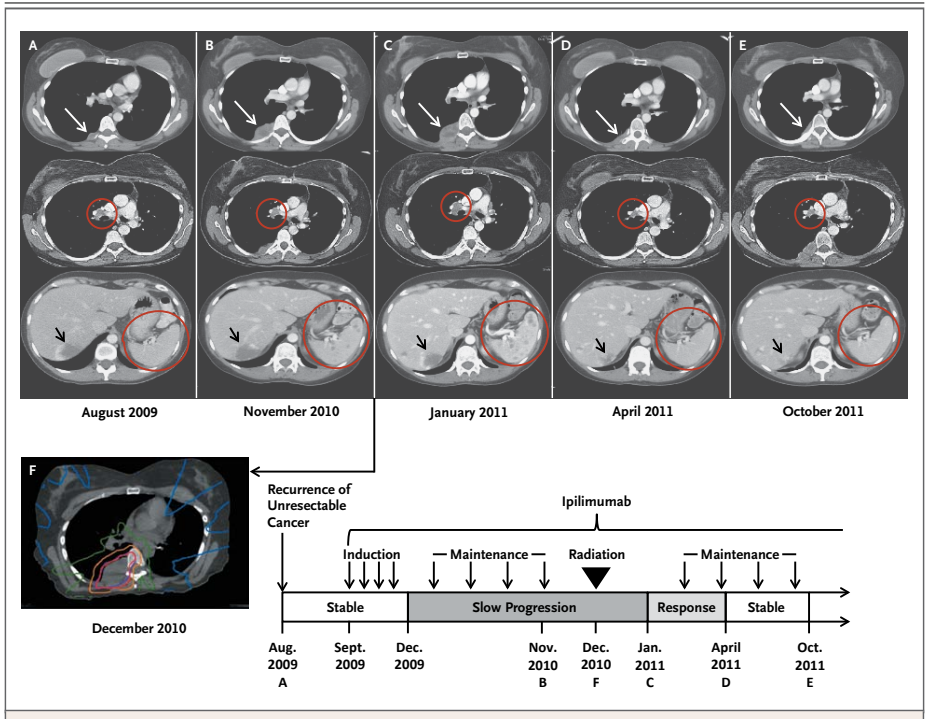
The signals from a few radionuclide labeled monoclonal antibody may be amplified by bystander signals within tumors and may also have long-range-abscopal or systemic effects



Tumor heterogeneity and non-linear dose response may lead to longer range, **abscopal** or systemic effects

Molecular pathways and targets outside directly exposed fields could contribute to a therapeutic response

Abscopal Effect



Abscopal effect is a phenomenon in which local radiotherapy is associated with the regression of metastatic cancer at a distance from the irradiated site

The patient had a response in hilar nodes and spleen after localized radiotherapy to paraspinal mass while receiving ipilimumab

Postow 2012; NEJM 366:925-931

Lecture Outline

- Reproductive Integrity
- Mechanisms of Cell Killing
- The *In Vitro* Survival Curve
- The Shape of the Survival Curve
- Appendix – Bystander Effect
- **Review Questions**

Question 1

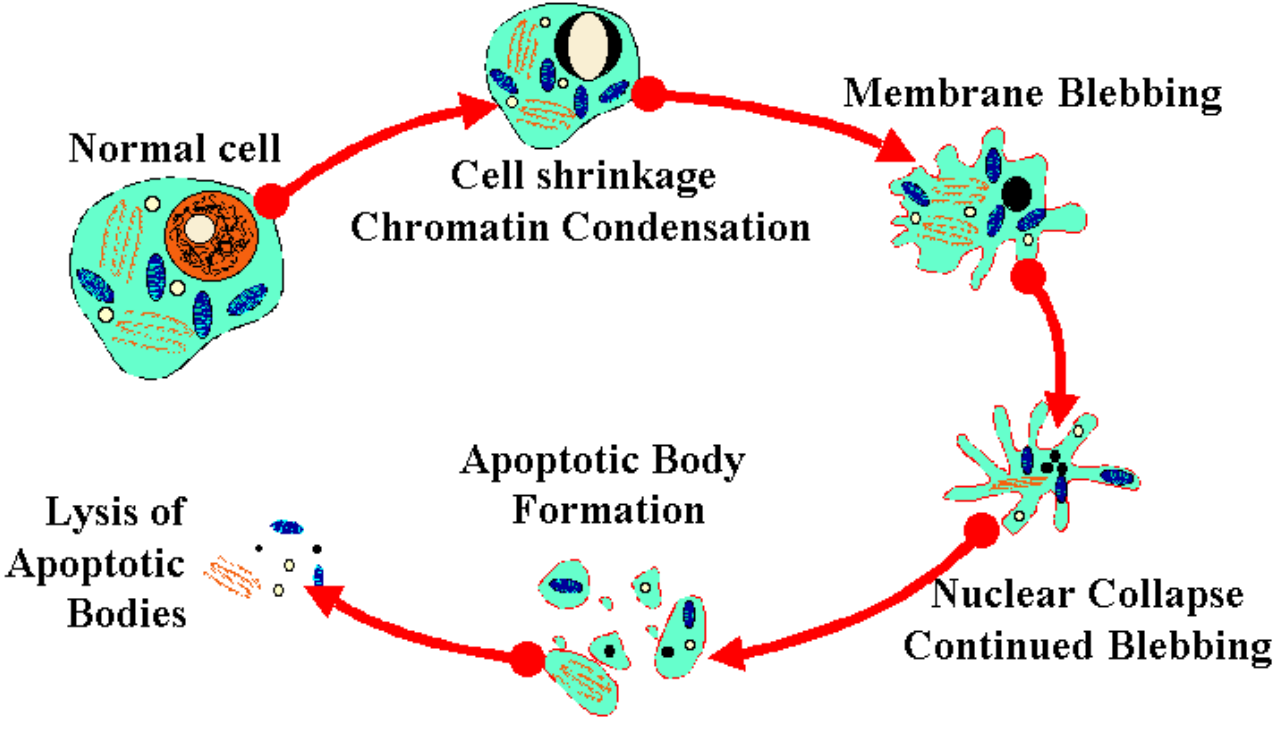
Morphological and biochemical features of apoptosis include all of the following, EXCEPT:

A. DNA cleavage
B. cell shrinkage
C. condensation of chromatin at the periphery of the nucleus
D. requirement for ATP
E. rupture of the plasma membrane

Apoptosis

Morphologic Hallmark = condensation of the nuclear chromatin

Biochemical Hallmark = DNA fragmentation



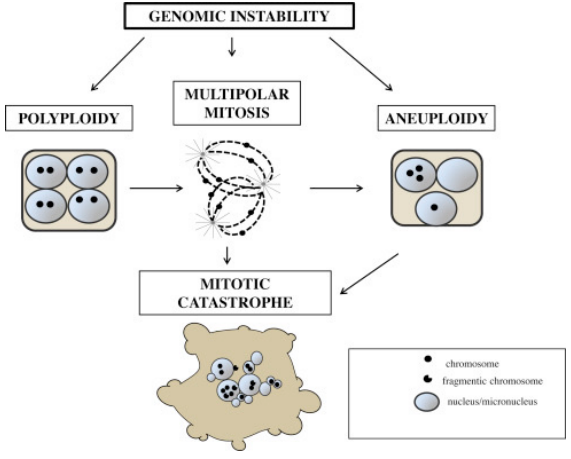
Question 2

Mitotic catastrophe following irradiation is a consequence of:

A. the presence of chromosome aberrations that interfere with cell division
B. G1 arrest
C. a reduction in cellular ATP levels
D. radiation-induced senescence
E. inactivation of tumor suppressor genes

Mitotic Catastrophe

- Mitotic catastrophe results from aberrant mitosis and can produce giant, multinucleated aneuploid cells that remain metabolically active
- Mitotic catastrophe is associated with deficiencies of the G2 and mitotic spindle checkpoints
- Often such cells will fail in the final stage of karyokinesis (nuclear cleavage) and cytokinesis (cellular cleavage) which results in giant cells reforming a single nuclear envelope with tetraploid DNA content and double the normal G1 chromosome number
- Cells undergoing mitotic catastrophe may subsequently die by apoptosis and mitotic cell death, suggesting that mitotic catastrophe may not be a specific cell death program but precedes other modes of cell death



Question 3

Radiation induced bystander effects

A. are associated with methylation
B. appear in non-irradiated cells cultured in the presence of irradiated cells
C. result in hypersensitivity to subsequent radiation exposure
D. are associated with radiation-induced hypoxia
E. are associated with radiation-induced hyperthermia

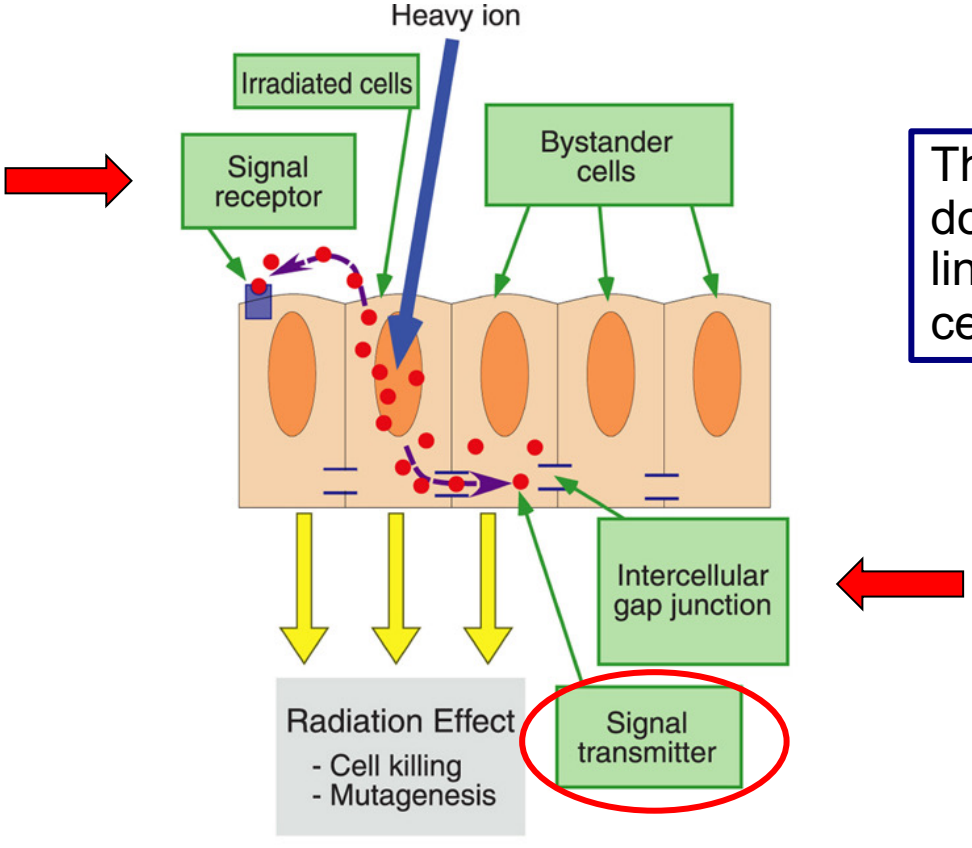
The Bystander Effect

- In addition to direct damage to DNA, a ***bystander effect*** has also been implicated in various radiation-induced biological effects, including chromosomal aberrations and cell killing

Bystander Effect = the induction of biologic effects in cells that are ***not directly*** traversed by a charged particle, but are ***in close proximity*** to the cells that are

- The bystander effect has been documented in both cancer cell lines and normal, untransformed cells

The Bystander Effect



The bystander effect has been documented in both cancer cell lines and normal, untransformed cells

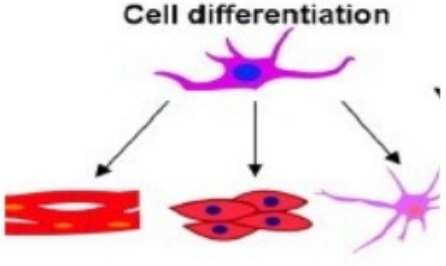
Question 4

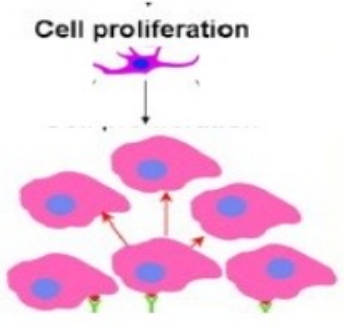
Which of the following methods would represent the best way to assess the radiosensitivity of actively dividing cells following irradiation?

A. clonogenic survival
B. division delay
C. trypan blue uptake
D. giant cell formation
E. detection of necrotic cells

Definition of Cell Death

- Cell death may mean different things in different context

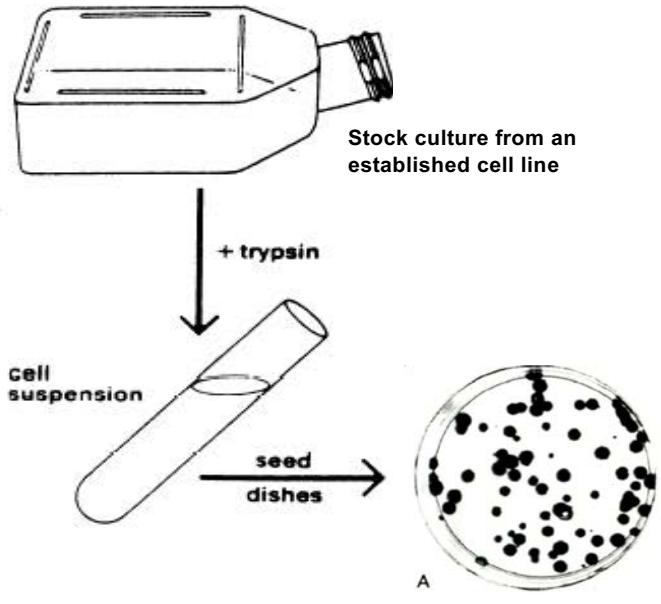




- For **differentiated cells** that do not divide (e.g., nerve, muscle), death can be defined as the loss of a specific function
- **For proliferating cells (e.g., bone marrow stem cells, intestinal epithelium, tumor), death can be defined as loss of the capacity for sustained proliferation, i.e., loss of *reproductive integrity***

Clonogenic Survival Assay

- Cells from an actively growing stock are harvested by gentle scraping or by the use of trypsin
- The number of cells per unit volume is determined
- Known numbers of cells are plated into fresh dishes
- If allowed to incubate for 1-2 weeks, clonogenic cells will form macroscopically visible colonies that can be fixed, stained and counted



Question 5

In a cell survival experiment with Chinese Hamster cells cultured *in vitro*, 100 unirradiated cells were seeded and allowed to grow for seven days before colonies were fixed and stained for counting. 80 colonies were counted. In a second group, 1000 cells that had been irradiated to a dose of 5 Gy were seeded and 40 colonies counted. The cell surviving fraction (SF) after 5 Gy was

A. 0.8
B. 0.5
C. 0.4
D. 0.05
E. 0.04

100 unirradiated cells yielded 80 colonies. Therefore, the plating efficiency of the cells is 0.8. Thus, the surviving fraction following 5 Gy is 40/(1000)(0.8) = 0.05

Question 6

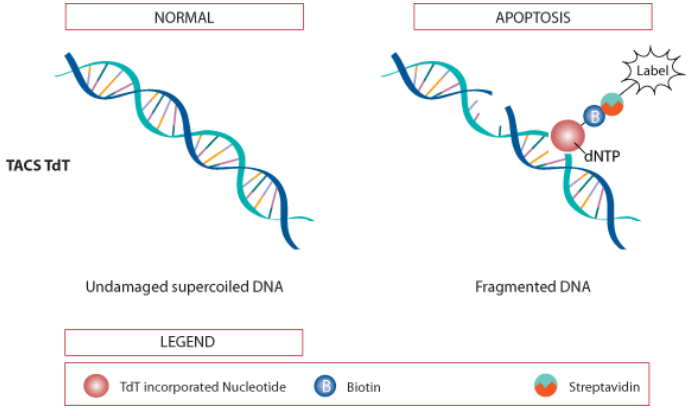
Medical Residents Only

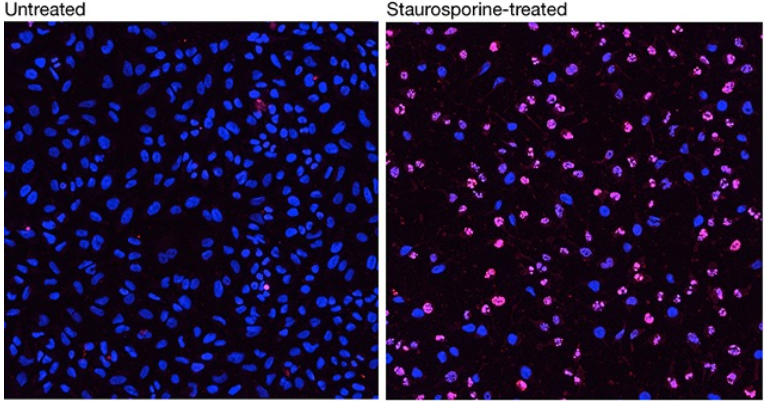
Which of the following would NOT be a useful assay for the detection of cells undergoing apoptosis?

A. TUNEL
B. DNA ladder formation
C. Annexin V labeling
D. DAPI
E. Staining with pimonidazole

TUNEL Essay

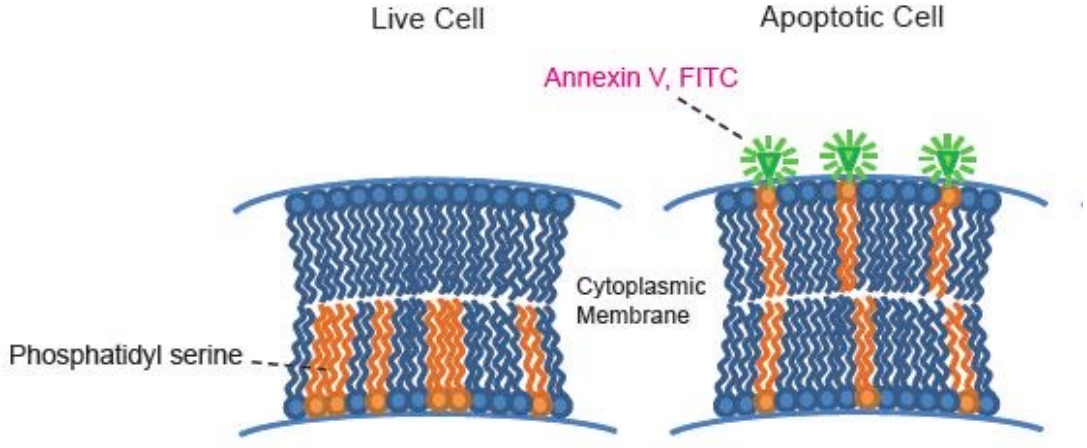
- The TUNEL (TdT-mediated dUTP Nick-End Labeling) technique has been widely used to identify apoptotic cells in many organisms
- Terminal deoxynucleotidyl transferase (TdT) is used to label 3′ hydroxyl DNA ends with modified nucleotides detectable by fluorescence or immunohistochemistry
- TUNEL specifically labels dying cells, which have more DNA breaks than viable cells as a consequence of DNA degradation





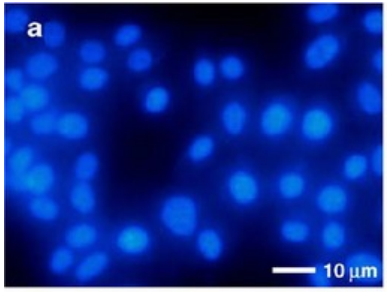
Annexin V Labeling

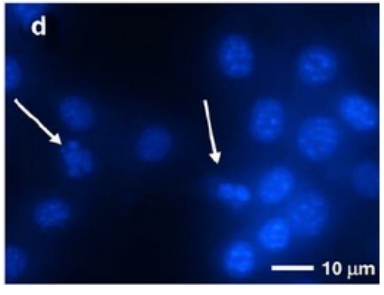
- Annexin V, is a protein that binds certain phospholipids called phosphatidylserines, which normally occur only in the inner, cytoplasm-facing leaflet of a cell's membrane, but become "flipped" to the outer leaflet during the early stages of apoptosis.



DAPI

- Diamidino-2-phenylindole (DAPI) is DNA-specific dye that displays a blue fluorescence.
- This dye could be used to assess the nuclear morphology of normal versus apoptotic cells by fluorescence microscopy.





Arrows represent the condensed or fragmented nuclei of cells.