Illinois Institute of Technology

Radiation Biophysics
Radiation Biology of Tumor Cells
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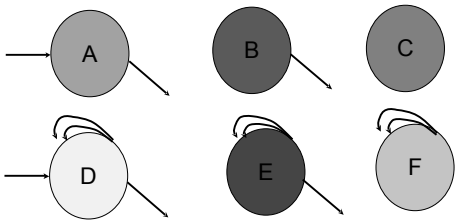
Class Plan

- Cell Death
- Cell population kinetics and cell survival
- Definition of Tumor
- How Tumors respond to Radiation
- Break
- Tools for studying tumor response
- Radiobiological Responses
- Hypoxia and radiosensitivity
- Dose fractionation and tumor therapy

Cell Death

- Clonogenic cell death:
  inability to produce several generations' worth of progeny
- Acute pathological cell death: necrosis
  - Cells typically swell, then lyse
  - Accompanied by inflammation
- Apoptosis
  - Programmed cell death
  - Shrinkage, fragmentation, phagocytosis
  - p53 is activator of genes that regulate it

Gilbert & Lajtha's cell types



Cell populations in Tissue

- A. Simple transit population
  - Cells in, cells out
  - Spermatozoa, blood cells
- B. Decaying population (e.g. oocytes)
- C. Closed, static population (neurons?)
- D. Dividing, transit population
  - Some cell division, so more leave than enter
  - Differentiating blood cells
- E. Stem cell population (many kinds)
- F. Closed, dividing population
  - No cells in or out—just a lot of division
  - Tumors, eye-lens epithelial cells

Cell population kinetics

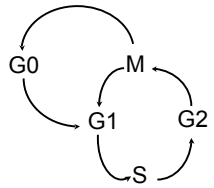
- Cell types that divide are the most sensitive.
- Cells are most sensitive during G2 and M, so cells that spend a lot of time in G2 and M are more sensitive
- If a cell population is exposed to radiation, the outcome depends on there being an adequate number of (clonogenically) surviving cells.

Growth Fraction

- Lajtha (1963): described "G0" phase in cell cycle: cell is not engaged in proliferation but could later re-enter proliferative stage
- Growth fraction is defined as fraction of total cellular population that is clonogenically competent and actually *in* the process of DNA replication and cell division.
- Measurement: uses ^{3}H-thymidine uptake
- Significance: cells in G0 have time to repair DNA damage
  - Works even if [repair enzymes] is low during G0
  - This is suspected but not proven

The expanded cell cycle (Lajtha)

- G0 is seen as an alternative to normal cycling
- Cells may re-enter the cycle after a change in environmental conditions or upon receiving a signal



What is a Tumor?

- A *tumor* is an mass of undifferentiated or poorly differentiated tissue growing amidst differentiated tissue.
- A tumor may be *malignant*, i.e. growing uncontrollably and with a propensity for spreading to other tissues.
- Or it may be benign, i.e. growing slowly or not at all and without a propensity for spreading
- The phenomenon of spreading is called *metastatis*.

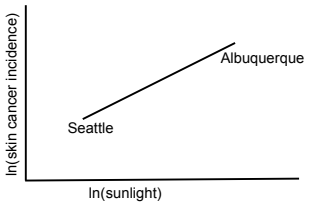
Cancer

Cancer is the growth of one or more malignant tumors. The process by which cancer develops is called *carcinogenesis*.

The causes, rapidity of onset, course of disease, treatment possibilities, and likely outcomes of cancer depend enormously on what tissue is being attacked, i.e. on the kind of cells from which grew.

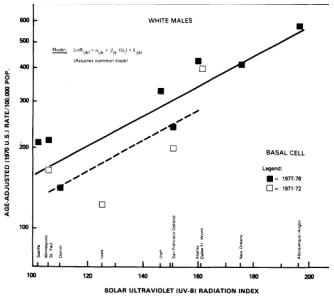
Skin Cancer and Sunlight

- Power-law relationship between skin cancer incidence and insolation



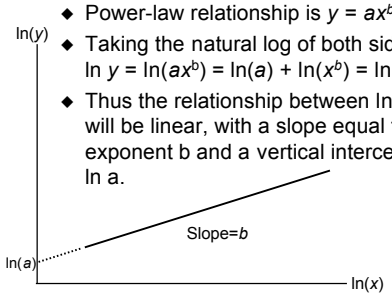
Actual study of this relationship

- Scotto, J, *et al*, (1983) NIH Pub. no. 83-2433.



Note about power laws

- Power-law relationship is $y = ax^b$
- Taking the natural log of both sides, $\ln y = \ln(ax^b) = \ln(a) + \ln(x^b) = \ln(a) + b\ln(x)$
- Thus the relationship between ln *y* and ln *x* will be linear, with a slope equal to the exponent b and a vertical intercept equal to ln a.

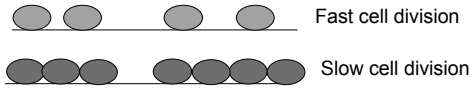


Characteristics of Cancer Cells

- Cancer cells lack differentiation
- Cancer cells have abnormal nuclei
  - May have an abnormal number of chromosomes
  - Gene amplification (abormal # of copies of specific genes) is common
  - Not subject to apoptotic controls
- Cancer cells form tumors
- Cancer cells metastasize

External Controls Exerted on Cells

- Hormonal and receptor-based controls
  - Apoptotic signals
  - Signals indicating entry or departure from G0
  - Signals enabling progression through the normal cycle
- Contact inhibition



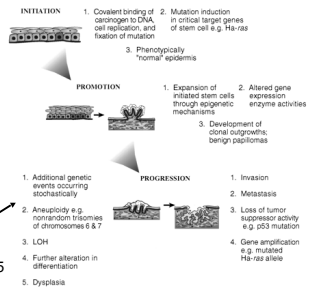
Cell types and cancer

- Epithelial cells (skin, digestive tract, tracheal lining, glands, . . .) give rise to *carcinomas*.
- Connective tissue cells (bone, cartilage) give rise to *sarcomas*.
- Blood cells give rise to *leukemia*.
- Lymphatic tissue gives rise to *lymphomas*.

Time-Course of Cancer

- Steps in causation:
  - Initiation
  - Promotion
  - Progression
- Steps in clinical outcome:
  - Exposure
  - Latency
  - Onset
  - Disease

Hursting, et al, (1999) JNCI 91:215

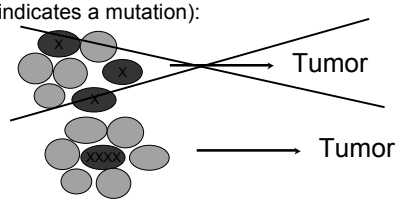


Initiation

- Initiation is typically a series of mutational events, often single-base changes in DNA in a single cell.
- The clonal hypothesis states that cancer typically arises from clonal growth out of a single damaged cell.
- In most cases it does appear that the number of mutations that have to occur in order for a tumor to grow out of a single cell is more than one.
- Initiation events can arise over a short time span if the exposure to the mutagen is intense and short (or if only one event is required).

The clonal hypothesis

- Hypothesis is that a tumor arises by clonal growth from a single multiply-mutated cell, rather than from several singly-mutated cells (here "X" indicates a mutation):

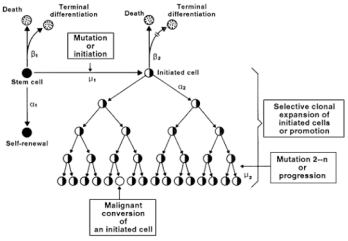


Promotion

- Promotion is a process in which metabolic and then morphological changes in the mutated cell occur.
- It does not typically involve mutations in the affected cell, but rather interference with some of the surveillance mechanisms by which these metabolic and morphological changes are controlled.
- Among the systems involved are the arachidonic acid cascade, by which the cell's differentiation capacity is regulated; and apoptosis factors like p53.

Promotion in Context

- James E. Trosko (1992), *RERF Update* **4**:3



Cell Kinetics

Attribute	Tumor	Growing cells
◆ *Total cycle time*	~20 hrs	~20 hrs
◆ *Time spent in S*	~8hrs	~8hrs
◆ *Vascularization*	chaotic	orderly
◆ *G0↔cycling transitions*	nutrient-dependent	regulated

Modeling Sensitivity in Tumors

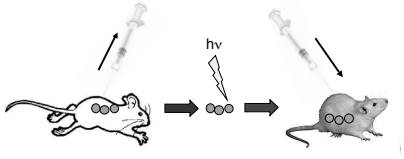
- Why?
  Because it enables us to optimize treatment regimens when exposing patients to radiation
  - Maximize cell killing in the tumor
  - Minimize damage to normal tissues
- Also provides test-bed for understanding interactions between tissues and radiation in general

Hewitt Dilution Assay

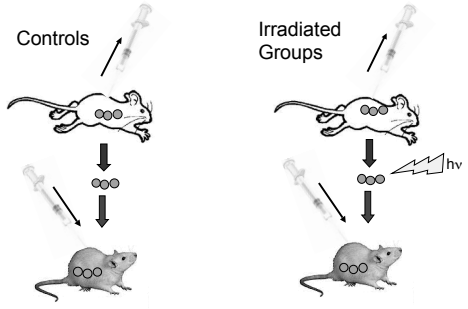
- Tumor cells grown in peritoneal (gut) cavity of mouse--"ascites" tumor
- Tumor cells can be harvested and injected into recipient mice
- Inject varying number of tumor cells and fraction killed against number of cells injected
- Result: if you pre-irradiate the tumor cells, they don't kill as many hosts.

Hewitt Procedure

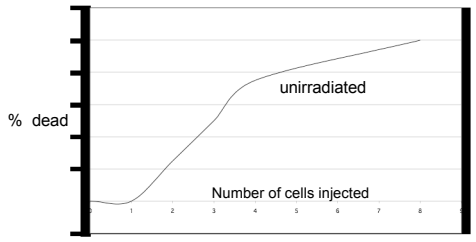
- Withdraw a few tumor cells from donor mouse
- Irradiate withdrawn cells in vitro
- Inject cells into target mouse



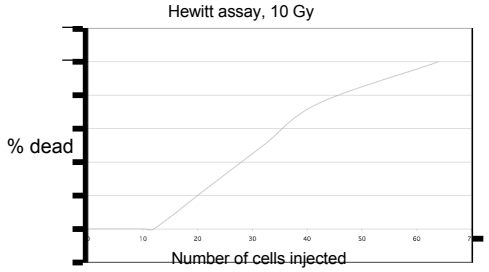
Control and Irradiated groups



Hewitt assay



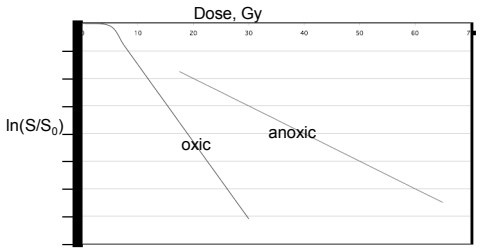
Hewitt assay, continued



Hewitt assay: analysis

- LD_{50} is used to construct survival curve
- In our example, 3 cells are enough to kill the host if no radiation is used; 32 are required if 10 Gy are used
- Evidently S/S_0 = 3/32 = 0.094 of the initial cells were functional enough to kill the host.
- We can calculate similar numbers for each dose level and calculate a dose-response curve (dose vs. $\log(S/S_0)$, perhaps under multiple conditions.

Hewitt survival curves



Lung Colony Assay

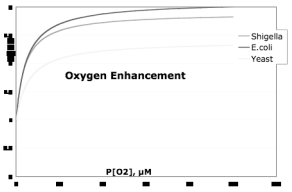
- Tumor is injected into a recipient mouse's lung
- Number of tumor colonies in target is counted
- Tumor may be irradiated:
  - In vivo
  - After dissection and cell dissociation
- Linear relationship between number of cells injected and number of colonies counted.
- 10-50X enhancement in # colonies if heavily irradiated, nonclonogenic cells are injected
- Irradiation increases # cells required to produce a given number of colonies

OER in Hewitt Assay

- Remember that OER is defined as ratio of dose required to get a given effect in the absence of oxygen to the dose required in the presence of oxygen. Since you need more dose to get the same effect if oxygen is absent, the OER is greater than one. For the data in fig. 10.3, the OER is about 2.2, since we need about 40 Gy of dose to damage anoxic cells the same amount as would require only 18 Gy with oxic cells. (40/18=2.2)

Enhancement as function of oxygenation

- Most of the oxygen dependence of cell survival happens at very low oxygen concentrations
- The difference between survival at 1% O_2 and 19% O_2 is minor

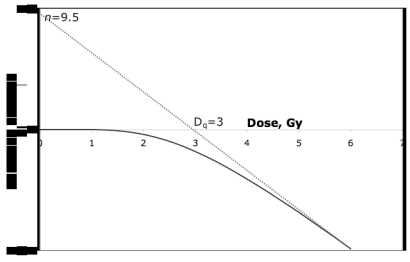


Lung Colony Assay

- Tumor is injected into a recipient mouse's lung
- Number of tumor colonies in lung is counted
- Tumor may be irradiated:
  - In vivo in the donor mouse
  - After dissection and cell dissociation but before injection
- Linear relationship between number of cells injected and number of colonies counted
- 10-50X enhancement in number of colonies if heavily irradiated, nonclonogenic cells are injected
- Irradiation increases the # of cells required to produce a given number of colonies

Lung Colony Assay Results

- KHT transplantable sarcoma: MTSH kinetics



Time-course of Tumor Growth after Irradiation

The growth delay interval is the time between irradiation and the time that the tumor recovers its original volume

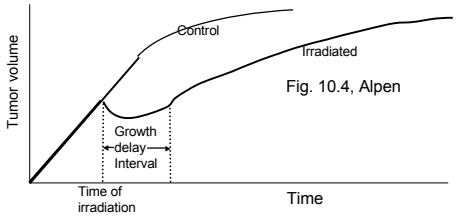
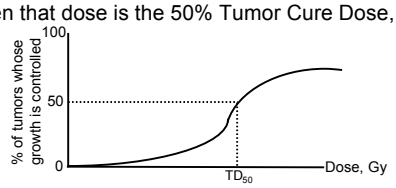


Fig. 10.4, Alpen

Tumor Cure Dose, TCD_{50}

To define the tumor cure dose, TCD_{50}:

- 1. Inoculate many animals with tumors
- 2. Irradiate them with a known dose
- 3. If this dose *controls the growth* of half the tumors, then that dose is the 50% Tumor Cure Dose, TCD_{50}.

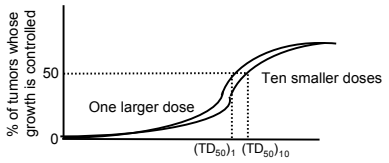


How tumors respond to radiation

- Transformed (immortalized) cell lines fairly similar
  - Reasonable agreement with MTSH kinetics
  - D_0 values range over a factor of 2-3 Gy
  - *n* values between 5 and 20
- Exceptions: DNA-repair-deficient cells
  - Xeroderma pigmentosum
  - Ataxia telangectasia
- Results from fresh tumors are somewhat different
  - Fit LQ models somewhat better than MTSH
  - 3 categories: high, medium, and low sensitivity
  - Correlation with responsiveness to radiotherapy

The influence of fractionation

- In many instances, the TCD_{50} for ten small doses separated in time is only slightly higher than for a single dose equal to the sum of the ten small ones
- The toxicity of the fractionated dose is much lower!



Tumor cells: mixed oxic and anoxic populations

We recognize kinetics characteristic of a mixture:

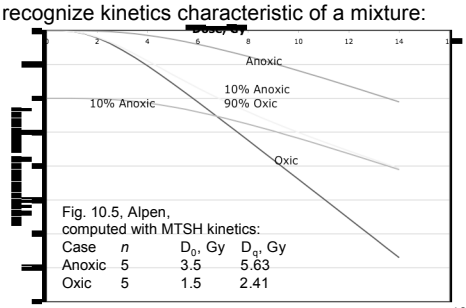
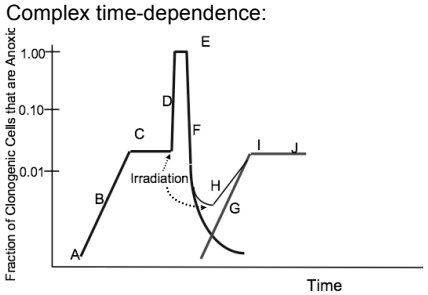


Fig. 10.5, Alpen, computed with MTSH kinetics:

Case	*n*	D_0, Gy	D_q, Gy
Anoxic	5	3.5	5.63
Oxic	5	1.5	2.41

Time-course of Anoxia

- Complex time-dependence:



Problems to consider

- Alpen, chapter 10, problem 1.
- Assume that ionizing radiation exerts its tumorigenic effects primarily through mutational events. Assume further that cigarette tar contains large numbers of cancer promoters. Which scenario would you expect would cause a higher incidence of cancer, and why?:
  - Irradiation followed by ten years of smoking
  - Ten years of smoking followed by irradiation