Illinois Institute of Technology

Radiation Biophysics
Lecture 12: Carcinogenesis
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Class Overview

- Tumors
  - Definitions
  - Prevalence and significance
  - Clonal theory
  - Multistage model
  - Systems for study
  - Human populations
  - Dose-response relationships
  - Latency
- Tumors (continued)
  - Absolute & Relative Risk
  - Where tumors happen
  - ED01 study: quantitative carcinogenesis
  - Latency
  - Absolute & Relative Risk
  - Differential Sensitivity
  - Locations of radiogenic tumors
  - Enzyme induction, revisited

Tumors: Definitions

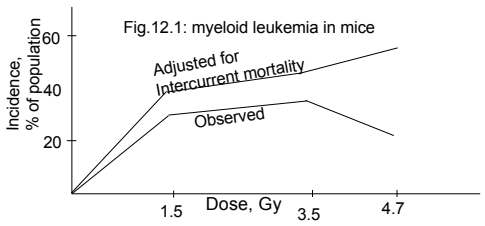
- Tumor: abnormal, de-differentiated cellular proliferation
  - Benign: small mass reaches a certain size and then stops growing
  - Malignant: those capable of uncontrolled growth & metastasis
- Cancer: a malignant tumor
- Carcinogen: a chemical or physical agent that increases the likelihood of cancer

Cancer: Prevalence and Significance

- 550,000 cancer deaths per year in the US
- 20-40% caused by environmental and workplace pollutants
- Others caused by smoking, diet, and natural causes
- Teasing apart these statistics is tricky:
  - Probability of any individual getting cancer under a particular set of circumstances is small
  - Multistage model makes multiple causes likely

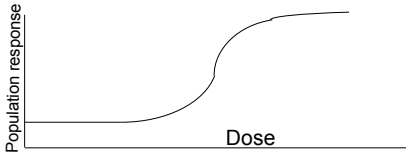
Tumors and Radiation

- Stochastic late effects (cf. end of last lecture)
  - Are these effects truly stochastic?
  - Even with cancer, there exists some dose-response effects in the individual



Tumors and Radiation (Cont'd)

- Is there a threshold?
  - Probably not (but is this a red herring?)
  - Not at the population level
- Serious Inquiry: the ED01 experiment
  Brown & Hoel, *Fundamental & Applied Toxicology* **3**: 458 (1983)



Upton's rules (remember?)

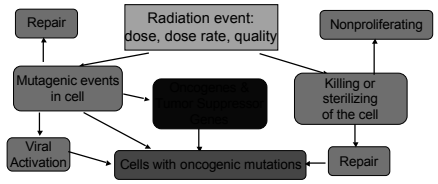
- Irradiation can produce almost any kind of neoplasm if we do it right
- Not every type of neoplasm has its incidence increased by irradiation of animals of any one species or strain
- Carcinogenic effects depend on a variety of mechanisms
- Some effects are direct, some are indirect
- Incidence rises more steeply with dose for high-LET radiation than for low-LET radiation
- Irradiation interacts with other causative agents
- Promotion may depend on other agents

How do Cancers Begin?: The Clonal Theory

- In general, mutational events in a single cell are sufficient to begin the process of tumorigenesis
- Often several mutations must arise in order for cancer to be a likely outcome
- Generally the mutation must be in one of the 50 or so genes that control cell replication and differentiation
- The mutagenic events are *never* enough to guarantee development of cancer
- Mutations must be followed by promotional events, which stimulate uncontrolled cell division

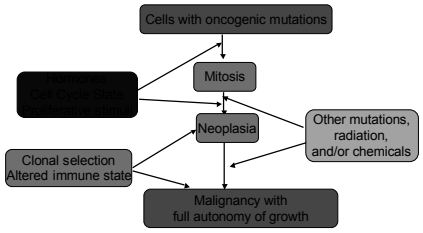
Events from transformation to mutated cells (fig. 12.2)

- Many factors influence events up through malignancy



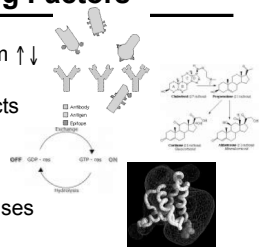
Mutations through Malignancy

- Additional influences seen



Modifying Factors

- Immune system ↑↓
- Hormonal effects
- Oncogenes
- Oncogenic viruses
- Environmental factors



How Cancers Develop: The Multistage Theory

- Initiation
  - DNA damage
  - e.g. Intercalators
- Promotion
  - Generally not mutational
  - Involves changes in control systems, e.g. arachidonic acid cascade
  - Tumors are present and capable of metastasis but haven't necessarily metastisized
- Progression
  - Development of metastatic tumors

Potentiation of Effect of Radiation by Smoking

- Inquiry into lung-cancer incidence among uranium miners and nearby office workers. Smokers and nonsmokers were surveyed.

		Uranium Exposure: Yes	Uranium Exposure: No
Smoking	Yes	+++	+
	No	—	—

How do we study radiation-induced carcinogenesis?

- Induction and progress of cancer in experimental animals
- Transformation of cells grown in tissue culture
- Human epidemiological studies
  - Accidental exposures: Radium-dial workers, Chernobyl victims, foot fluoroscopes
  - Medicinal exposures
  - Atomic bomb victims

What Constitutes a Cancer?

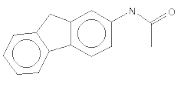
- Morphological change
- Cell immortality (escape from apoptosis)
- Tumorigenicity, i.e. spread of undifferentiated cells

Oncogenes

- Genes that are activated or show enhanced expression in tumors
- Limited data showing connection between human radiation-induced tumors and activation of oncogenes

ED01 study

- We mentioned this a bit earlier
- Study run by scientists at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina
- BALB-C mice analyzed for liver tumors
- Test compound was 2-acetylaminofluorene, a known carcinogen in rodents:



ED01 study, continued

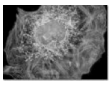
- 24000 mice in various exposure groups
- Endpoints and elements of study:
  - Time to tumor incidence
  - Dose “fractionation” (but this is a chemical)
- Sophisticated statistical analyses:
  - Initial analyses around 1981
  - Re-analysis a few years later
- Compared various dose-response models

ED01 quantitation

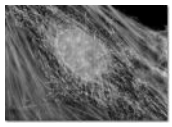
- Analyze tumor incidence according to $P(t,d) = 1 - \exp(-F(t,d))$, where t = time and d = dose.
- P, the tumor incidence fraction, behaves like 1-S in our survival curve studies
- Some analyses suggest that 2-AAF is primarily a promotor, not an initiator
  - So it isn't a great model for what radiation does…
  - But it still illustrates the importance of careful statistics!

Experimental Systems for Studying Rad-induced Tumors

- We need these because we can't deliberately do high-dose experiments on humans!
- CHO cells
  - Chinese Hamster Ovary
  - Good for looking at early effects--Initiation
  - Difficult to model the promotional events.
  - Transformation results in loss of contact inhibition

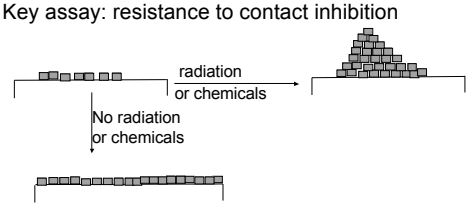


- Mouse embryo fibroblasts
  - Immortalized
  - Still display contact inhibition



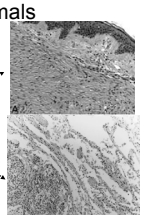
CHO Cells (Cont'd)

- Key assay: resistance to contact inhibition



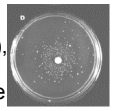
Mouse Embryo Cells:

- Experiment: growing total confluence
- Lose contact inhibition?
- Can induce tumors in syngeneic animals
- Limitation in both systems:
  - Fibroblasts (mesenchymals)
  - Most human tumors are epithelial



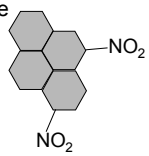
Mutagenesis

- Many chemicals, as well as radiation, can be shown to cause mutations.
- It's therefore logical to test for mutagenicity as a first-stage inquiry into the likelihood that a compound or a radiation treatment might be carcinogenic
- Standard mutagenic test: The Ames test (developed by Bruce Ames), which *Salmonella* cells are exposed to a chemical and mutation rates in the cells are measured.



Is an Ames Test a Good Substitute for These Complex Systems?

- No!
- 1,8-dinitropyrene is the most mutagenic substance known in the Ames test; yet it is only weakly tumorigenic in rats.



Why might we care about dinitropyrene?

- Most mutagenic substance known in *Salmonella* strain TA98: 72900 revertants/nanomole
- Nitroarenes like this one were found to be present in used toner, i.e., combustion waste from Xerox toner
- When this appeared, Xerox chemists reformulated their toner to drastically reduce the nitroarene content in the used toner.
- Mermelstein (1981) *Mutation Research* **89**:187-196.
- Löfroth et al(1980) *Science* **209**:1037-1039 and Mermelstein et al (1980) *Science 209*:1039-1043.
- So: all's well that ends well!

This is also a story about enzyme induction

- Nitroarenes like dinitropyrene and other polynuclear aromatic hydrocarbons, (e.g. benzo[a]pyrene) are known to be inducers of enzyme activities
- Some of these enzyme activities actually activate toxicants rather than detoxifying them
- Most of the activity of these enzymes will detoxify;
- But if 1% makes things worse, we want to understand that 1% activation
- So we found that pretreatment with these compounds could induce subsequent binding of other compounds to mouse DNA:
  Howard et al (1986), *Biochem. Pharm.* **35**: 2129-2134.

Animal Cell-Line Cancer Studies

- How similar are these rodent cell systems (CHO, mouse) to human cells?
- Answer: Human cells:
  - Are more resistant to spontaneous immortalization
  - Tend to give more nearly linear responses to dose
  - Radical scavengers and cold don't protect as much:
    That suggests that direct mechanisms prevail in humans and indirect mechanisms are more important in rodents

More on humans vs. rodents

- High-LET studies indicate that repair is less effective in humans
- Thought: why might rodents have more proficient repair systems than humans: is there an evolutionary lesson there?
- Promotion can be studied in animal cells, along with initiation

Radiation Carcinogenesis in Human Populations

- Occupational: radiologists, miners, dial painters
- Medical exposures:
  - Ankylosing spondylitis
  - Nonmalignant disease in pelvis and breast
  - Multiple fluoroscopies to chest (e.g. in TB patients)
  - Infants & children with enlarged thymus and ringworm
  - Children exposed in utero to diagnostic X-rays
- Nuclear accidents and weapon detonations
- Environmental background (see last chapter)

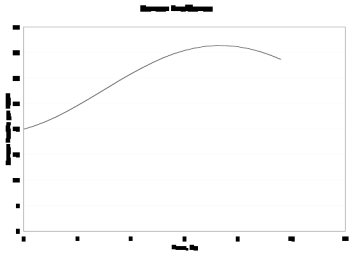
Dose-Incidence in Cancer Studies

- We seek a relationship relating post-exposure incidence I_D to dose D and normal incidence I_n
- Model might be:
- Linear: $I_D = I_n + \alpha_1 D$
- Quadratic: $I_D = I_n + \alpha_2 D^2$
- LQ: $I_D = I_n + \alpha_1 D + \alpha_2 D^2$
- Corrected for loss of clonogenic potential:
  $I_D = (I_n + \alpha_1 D + \alpha_2 D^2)\exp(-\beta_1 D+\beta_2 D^2)$

Corrected models

- Graphical results of corrected model (with $\exp(-\beta_1 D+\beta_2 D^2)$ term included):

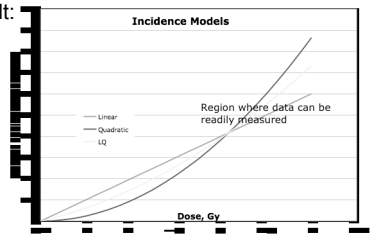
$\alpha 1 = 2\ Gy^{-1}$
$\alpha 2 = 0.8\ Gy^{-2}$
$\beta 1 = 0.03\ Gy^{-1}$
$\beta 2 = -0.01\ Gy^{-2}$



Linear, Quadratic, LQ Models

- We try to devise low-dose models based on high-dose data, where the three models are close together. It's often difficult:

(my version of fig. 12.5)



Latency

- Definition (in the cancer context):
  Time between the mutational events that began cellular transformation and the appearance of a medically observable malignancy
- How long in humans?
  - A few years (blood or lymphatic cancers)
  - 15-30 years for solid tumors
  - Animals: scale these numbers to animal's lifespan
  - These numbers are minima:
    leukemia *can* take > 15 yrs,
    even though it's typically listed as having ~ 5yr latency

Latency, revisited

- Cancer takes a long time to arise
- Causes:
  - Often we need several mutations to arise
  - Promotion involves gradual exposure to promotional agents
  - Cell turnover
- Confusing:
  - Why are there still excess leukemia cases in Hiroshima even though the latency is short?
  - Requires careful correction for other forms of mortality to get meaningful data (e.g. smoking)

Absolute & Relative Risk

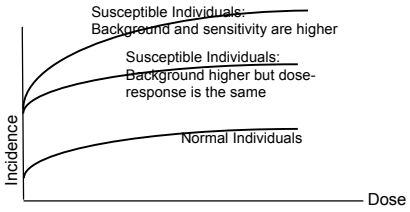
- Does a change in background incidence influence the dose-dependent incidence?
- Absolute risk:
  - Dose-dependent risk is independent of spontaneous (non-dose-dependent) risk
  - $I_D = I_n + f(D)$ such that $f(0) = 0$.
- Relative risk:
  - Likelihood of radiogenic cancer is related to natural incidence
  - Risk is multiplicative of spontaneous risk
  - $I_D = I_n * g(D)$, where $g(0) = 1$.

Absolute & Relative Risk: Math

- Absolute: $I_D = I_n + f(D)$ such that $f(0) = 0$
- Relative: $I_D = I_n * g(D)$, where $g(0) = 1$
  - Take the natural log of both sides:
  - $\ln(I_D) = \ln(I_n * g(D)) = \ln(I_n) + \ln(g(D))$,
  - so for $q(D) = \ln(g(D))$, $J_D = \ln(I_D)$, $J_n = \ln(I_n)$,
  - $J_D = J_n + q(D)$, $q(0) = 0$,
  - which looks a lot like the absolute risk model with slightly different variables!

Differential Sensitivity and Dose-Incidence Relationships

- The background and the dose-dependent response may be different for hypersensitive individuals



So which is correct— Absolute or Relative Risk?

- Very difficult to sort out
- Data are shaky
- Need to find ways to change
- Animal studies:
  most data favor relative-risk model
- Human studies:
  most people accept relative-risk model

Excess Cancer Deaths for the two Models

Does the choice of additive vs. relative matter much in projecting risk? Yes (table 12.1):

Non-leukemia deaths	Continuous lifetime exposure, 1mGy/yr (deaths/100,000)		Instantaneous exposure, 0.1 Gy (deaths/100,000)	
	Males	*Females*	*Males*	*Females*
BEIR III, Additive	24.6	42.4	42.1	65.2
BEIR III, Relative	92.9	118.5	192	213
BEIR V, Relative	450	540	660	730

Where in the body do radiogenic cancers appear?

- Tricky to study because of latency
- Bomb results for acute exposure ~ 1 Gy, low LET:

Deaths/10^5

Type	*Multiplicative Model*	*Additive Model*	*Type*	*Multiplicative Model*	*Additive Model*
Leukemia	97	93	Ovary	31	26
Bladder	39	23	Esophagus	34	16
Breast	60	43	Stomach	126	86
Colon	79	29	Remainder	114	103
Lung	151	59			
Multiple myeloma	22	9	*TOTAL*	*707*	*453*

Enzyme induction, revisited

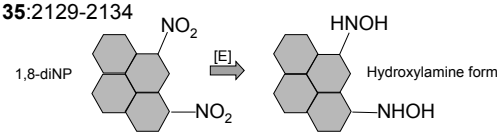
- Enzyme induction is relevant to radiation-induced carcinogenesis and many other toxicological and biochemical contexts.
- Two examples:
  - Activation of chemical agents into metabolically active forms (the nitroarene story)
  - DNA repair enzymes activated by low doses of ionizing radiation (2003 results in *PNAS*, reported by two students in this course)

Enzyme induction: the principle

- Cells often synthesize enzymes on an as-needed basis.
- Why?
  - It's metabolically expensive to synthesize enzymes (or other proteins) that aren't needed
  - The enzymes may act on substrates that are not what the cell "wants" them to act on; they may thereby do damage
- Therefore: changes in environmental conditions give rise to changes in concentrations and identities of the enzymes that are synthesized via transcription and translation.

Enzyme induction with nitroarenes

- 1,8-dinitropyrene is enzymatically converted to a metabolically more active form. The enzyme involved can be *induced* by pretreatment of the animal with 1-nitropyrene, benzo(a)pyrene, or other agents.
- Howard et al (1986), *Biochemical Pharmacology* **35**:2129-2134



Enzyme Induction: Repair of Double-Strand Breaks in DNA

- Rothkamm & Löbrich (2003) *PNAS* 10.1073, i.e. *PNAS* **100**:5057-5062: DSB DNA repair after low doses of X-irradiation. Result:
- Low doses gave rise to slow DNA repair (days)
- Higher doses gave rise to rapid DNA repair (hours)

Human Cell Culture → Very low X-ray doses → *Several days before repair of radiation-induced DNA breaks.*

Human Cell Culture → Therapeutic X-ray doses → *Repair is rapid: most lesions are repaired in hours.*

So what's going on here?

- Authors of study are careful not to say that this indicates that high doses are better for you than low!
- Fidelity of repair may be a problem (DSBs!)
- How about suggesting that enzyme induction doesn't occur unless a certain amount of dose arrives?
- Perhaps there's a threshold for induced DSB enzymatic repair
- n.b. Thanks to Michael Mysz and Don Parry for bringing this to my attention!

Enzyme induction as a cause

- My suggestion (I read the paper a while ago…)
- Low-dose case: constitutive levels of DSB repair are low but nonzero and provide for slow DNA repair
- High-dose case: DSB repair enzymes induced fast!

Human Cell Culture → Very low X-ray doses (Only constitutive enzymes used) → *Several days before repair of radiation-induced DNA breaks.*

Human Cell Culture → Therapeutic X-ray doses (Induced enzymes participate vigorously) → *Repair is rapid: most lesions are repaired in hours.*

Would this be surprising? No.

- Many enzymes exist in both constitutive and inducible forms
- Nitric oxide synthase is a characteristic example
- Constitutive enzyme levels are sufficient to deal with routine problems
- Inducible enzymes appear (at nonzero metabolic cost) when the cell is stressed in characteristic ways.