Radiation Carcinogenesis

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Overview

- History of radiation and radiation-induced damage
- Bystander effect of radiation
- Methods for DNA damage analysis
- Stages of carcinogenesis and models
- Mechanism of radiation-induced carcinogenesis
- Role of oncogenes and tumor suppressors
- Risk projections and risk estimates
- Importance of dose and age on tumor incidence
- Second malignancy after radiotherapy
Radiation and cancer

- 1895- Roentgen discovered X-rays
- 1896- Becquerel discovered radioactivity
- 1897- Rutherford discovered α and β rays
- 1898- Curies discovered polonium and radium
- 1902- First report on radiation-induced cancer
- 1911- First report of leukemia in 5 radiation workers
Marie Curie and Her Daughter Irene —
Thought to have Died of Leukemia
Types of radiation

Ionizing radiation:
- $\alpha$ particles (2 protons and 2 neutrons)
- $\beta$ particles (electron equivalent)
- Neutrons
- Gamma rays
- X-rays

Non-ionizing radiation:
- Microwaves
- Visible light
- Radio waves and TV waves
- UV radiation (except shortest wavelengths)
Adapted from: “Ultraviolet light as a carcinogen”, Ananthaswamy, 1997
Units and doses

Activity:
Quantity of a radionuclide which describes the rate at which decays occur in an amount of a radionuclide.

The SI unit of radioactivity is the becquerel (Bq), which replaced the old unit, the curie (Ci).

Becquerel (Bq): One becquerel corresponds to 1 disintegration of a radionuclide per second.

Curie (Ci): Old unit of radioactivity, corresponding to $3.7 \times 10^{10}$ radioactive disintegrations per second
Units and doses

Absorbed dose (D): The energy imparted per unit mass by ionizing radiation to matter at a specific point.

Gy: The SI unit of absorbed dose is joule per kilogram (J kg\(^{-1}\)). The special name for this unit is gray (Gy).

Rad: The previously used special unit of absorbed dose, the rad, was defined to be an energy absorption of 100 ergs/gram. Therefore, 1 Gy = 100 rad.
Units and doses

Relative biological effectiveness (RBE) - A factor used to compare the biological effectiveness of different types of ionizing radiation. It is the inverse ratio of the amount of absorbed radiation, required to produce a given effect, to a standard (or reference) radiation required to produce the same effect.

Rem - Old unit of equivalent or effective dose. It is the product of absorbed dose (in rad) and the radiation weighting factor. 1 rem = .01 Sv.

Sievert (Sv) - SI unit of equivalent dose or effective dose. 1 Sv = 100 rem.
**Linear energy transfer (LET)**

- The rate of energy loss or deposition along the track of an ionizing particle

- Loss of energy/unit distance traveled in matter

- Units = KeV/µm

- Varies depending of quality of radiation
Linear energy transfer (LET)

x-ray or $\gamma$-ray: $\times \quad \times$ Sparsely Ionizing

$\beta$ particle: $\times \quad \times \quad \times$

Neutron: $\times \times \times \times \times \times \times$ Densely Ionizing

$\alpha$ particle $\times \times \times \times \times \times \times \times \times \times \times \times$

The more sparsely ionizing, the more penetrating
Radiation-induced cancer in human

- Atomic bomb survivors
- Accidents
- Medically exposed individuals including cancer patients undergoing radiation therapy
Early cases of human experience

• Skin cancer in early x-ray workers

• Lung cancer in underground uranium miners in Saxony and Colorado

• Bone cancer in radium dial painters

• Liver cancer in thorotrast patients
Later cases of human experience

- Hiroshima/Nagasaki survivors
- Anklyosing spondylitis patients
- Elevated incidence of leukemia in early radiologists ca 1922
- Thyroid cancer from treatment for enlarged thymus
- Thyroid and other cancers for treatment of tinea capitis
- Breast cancer in tuberculosis fluoroscopy patients
Radiation-induced chromosomal aberrations
Sources and consequences of DNA damage

Exogenous Sources
- UV and other radiation sources, chemicals

Endogenous Sources
- ROS, alkylation, hydrolysis

DNA damage leads to:
- Misreplication, aberrant chromosomal segregation
- Mutations, chromosomal aberrations
- Blocked transcription, blocked replication
- Cell-cycle delay or arrest, cell death
- Cancer
- Aging

DNA repair systems
DNA adducts as markers of exposure in human

- Early event in the overall process leading to carcinogenesis
- Measure of the internal dose or “biologically effective dose”
- Adducts in target tissues - correlated with cancer status
- Easily measured in surrogate tissues (e.g., lymphocytes)
- Correlated with genetic risk factors (polymorphisms in DNA repair & drug metabolizing genes).

- The nature of DNA adducts can give clues to the etiological agent.

- Mutagenicity of DNA adducts depends on (i) the type of DNA adduct formed, (ii) extent to which it is repaired, (iii) its sequence context and (iv) the polymerases involved.
Methods for DNA adduct analysis

- $^{32}$P-Postlabeling
- High performance liquid chromatography (HPLC)
- HPLC with electro-chemical detection (HPLC-ECD)
- Immunohistochemistry (IHC)
- Enzyme-linked immunosorbent assay (ELISA)
- Radio-immuno assay (RIA)
- Gas Chromatography/Mass Spectrometry (GC/MS)
- Single cell gel electrophoresis (Comet assay)
- Fluorescence Spectroscopy
- Slot-blot analysis
Measurements of DNA damage
Measurements of DNA damage

(a) Images showing control and irradiated cells over time.

(b) Graph showing the number of γ-H2AX foci post-irradiation.

(c) Graph showing DNA damage over time post-irradiation.

(d) Graph showing the percentage of DNA DSBs post-irradiation.

Goutam et al., Int J Radiat Biol Phys. 2012, July 24
Measurements of DNA damage

Undamaged cell

Cell irradiated with 12.5 Gy X-rays

Cell treated with a DNA cross-linking agent and irradiated with 12.5 Gy X-rays

Hartley et al., Cancer Cell
Culture: Methods and Protocol
Vol 731 Chapter 25
Major regulatory steps in the process of DNA damage response
DNA damage and cell death

H. Rodriguez-Rocha et al., Mutation Research 2011, 711, 158–166
DNA damage and apoptosis

- DNA damage
- ATM
- Chk1/2
- p53
- Mdm2
- ARF
- E2F
- p73
- Apaf1
- Caspase-9
- Caspase-3
- Apoptosis
- TopBP1
- SirT1
- PCAF/p300

Current Opinion in Cell Biology
DNA damage and autophagy

H. Rodriguez-Rocha et al., Mutation Research 2011, 711, 158–166
Radiation-induced chromosomal aberrations

X-rays or ionizing radiation induces DSBs in the chromosomes. DSBs causes sticky ends, which can join with any other sticky ends.

1) Rejoin to original configurations
2) The breaks fails to rejoin causing deletion
3) Broken ends may join other sticky ends
Acentric and dicentric chromosomes
Ring chromosome
Translocation, deletion, and inversion
Bystander effect

• Genetic alterations can occur in cells that receive no direct radiation exposure

• Damage signals transmitted from neighboring irradiated cells
Bystander effect

This Timeline shows how radiation-induced bystander effects were documented in the literature as early as 1954, but were not integrated into mainstream radiobiological studies until over 40 years later.
Bystander effect

Figure 2 | Key aspects of radiation-induced bystander responses. Typical dose response curves for direct (a) and bystander (b) responses are shown, highlighting the commonly observed saturation of response for bystander effects.
Bystander responses
Mouse skin model

1. Covalent binding of carcinogen to DNA, cell replication, and fixation of mutation.
2. Mutation induction in critical target genes of stem cells, e.g. H-ras
3. Phenotypically "normal" epidermis

- Initiation

1. Expansion of initiated stem cells through epigenetic mechanisms
2. Altered gene expression/enzyme activities
3. Angiogenesis

1. Production and maintenance of chronic cell proliferation
2. Development of clonal outgrowths; benign papillomas
3. Altered differentiation
4. Diploid stem line

- Promotion

1. Additional genetic events occurring stochastically
2. Aneuploidy e.g. nonrandom trisomies of chromosomes 6 & 7
3. LOH
4. Further alteration in differentiation
5. Dysplasia

1. Invasion
2. Metastasis
3. Loss of tumor suppressor activity e.g. p53 mutation
4. Gene amplification e.g. mutated Hras allele

GENETIC SUSCEPTIBILITY
• **Initiators**
  → Are chemically reactive or requires metabolic activation to chemically reactive intermediates.
  → React covalently with cellular macromolecules such as DNA, RNA, protein.
  → Produce an essentially irreversible event after a single application.
  → Are mutagenic in bacterial and mammalian cells.

• **Promoters**
  → Do not require metabolism, i.e., are active in their parent form.
  → Require prolonged and repeated exposure.
  → Actions are essentially reversible.
  → Produce biochemical and cellular responses typical of gene derepression.
  → Can be subdivided into at least two distinct stages: Stage I (conversion); and Stage II (propagation).
Overview of carcinogenesis

External Exposure → Metabolic Activation → DNA Damage → Fixation

Internal Exposure → Biologically Effective Dose → DNA Repair

Detoxification → Reactivation → Excretion

Initiation → Promotion → Progression → Preclinical Biologic Effect → Cancer

Latency Period

Exposure

~ 1 day

~ 20-40 yrs
Oncogene activation and inactivation of tumor suppression genes

- Activation of proto-oncogenes
- Loss of function of tumor suppressors
- Infection with certain viruses
- Substitution of normal promoters of proto-oncogenes with strong promoters of viruses
- Chromosomal aberrations
Oncogene activation and inactivation of tumor suppression genes

- Mutational event in initiation of radiation carcinogenesis most likely involves LOH of a tumor suppressor gene

- Deletion of RB tumor suppressor gene on 13q14

- Hypersensitivity of retinoblastoma patients to the induction of secondary cancers
Oncogene activation and inactivation of tumor suppression genes

- Knockout mice heterozygous for p53 tumor suppressor gene more susceptible to radiation induced tumors

- Expression of p53 mutations occur late in radiation-induced malignant transformation

- Activation of oncogene RAS family reported in mouse lymphomas
Oncogene activation and inactivation of tumor suppression genes

- Radiation may induce papillary thyroid carcinomas in children as a result of oncogene activation
- Amplification/overexpression of MDM2 found in X-ray transformed foci and expression of mutant p53
- Multiple pathways for transformation
### Occurrence of p53 mutations in UV-induced tumors

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Total mutation frequency (%)</th>
<th>% CC→TT</th>
<th>% C→T</th>
<th>% other</th>
<th>% non-pyr site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinic keratosis</td>
<td>58</td>
<td>2</td>
<td>60</td>
<td>19</td>
<td>19</td>
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<tr>
<td>Basal cell</td>
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<tr>
<td>Carcinoma (BCC)</td>
<td>49</td>
<td>12</td>
<td>38</td>
<td>16</td>
<td>34</td>
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<tr>
<td>Squamous cell</td>
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</tr>
<tr>
<td>Carcinoma (SCC)</td>
<td>45</td>
<td>11</td>
<td>29</td>
<td>32</td>
<td>29</td>
</tr>
</tbody>
</table>
Four-stage hypothesis

- Chromosomal damage in normal dividing cells
- Defect in differentiation genes
- Gene defect in hyperplastic cells
- Gene defect in cancer cells
Chromosomal damage in normal cells

- Low or high dose radiation exposure can lead to chromosomal damage in normal cells.

- These cells may undergo cell death, divide, or
Defect in differentiation genes

• One or two normal damaged cells develop a defect in differentiation genes, which prevent them from a normal pattern of differentiation and death.

• Continuing division of these cells leads to hyperplasia and develop in adenoma.
Accumulated gene defects in cells causes cancer

• One or two hyperplastic cells in any adenoma can accumulate additional gene defects due to mutations or chromosomal damage, which can make them cancerous.
Colon tumor model

Microsatellite instability
rate increases as tumor progresses

- c-ki-ras
- TGF-α
- c-myc
- APC
- DCC
- p53
- bcl-2 down regulation
- Mutations in DNA repair genes
- bax mutations
- TGF-β RII

NORMAL MUCOSA ➔ HYPERPROLIFERATION ➔ ADENOMA ➔ CARCINOMA ➔ METASTASIS

FIRST MUTATION ➔ SECOND MUTATION ➔ BENIGN TUMOR ➔ THIRD MUTATION ➔ ADVANCED TUMOR ➔ MORE COMPLEX MUTATION ➔ INVASION/METASTASIS

- Damaged normal cells
- Differentiation defective
- Accumulation of gene defects
- Complex gene aberration
UVA

Direct
- Type 1 photosensitisation (8oxoG)
- Triplet energy transfer (CPD)

Indirect (ROS-mediated)
- Type 2 photosensitisation
- Enzymatic ROS (e.g. NADPH oxidase)

Damage to biomolecules
- Damaged DNA bases (e.g. 8oxoG, CPD) and strand breaks
- Lipid peroxidation & Protein oxidation

Damage response
- Checkpoints G1/S, G2/M
- Stress response ATM, MAPK, p53?
- DNA Repair NER, BER, MMR, NHEJ

Apoptosis

Mutations, chromosomal aberrations

Malignant transformation

Cell survival

error-prone repair
UVB

Skin

Photochemopreventive Agents Act As

- Sunscreen (1st line)
  - Prevention of Damage
    - Gene inactivation
- Antioxidant (2nd line)
  - Radical scavenger
    - Oxidative stress
    - DNA damage
    - p53
      - DNA excision repair
- Redox regulation of signal transduction pathway (3rd line)
  - Inflammation
  - Proliferation
  - Transformation specific apoptosis in transformed cells

Prevention

Correction
UVB

Skin

Initiated cells

Clonal expansion

Tumor promotion

Critical targets

NF-κB

AP1

PI3K/AKT

MAPK

EGCG
Resveratrol
Silymarin
Glycolic acid
Vitamin E

EGCG
Theaflavins
perillyl alcohol
acetylsalicylic acid

EGCG
Theaflavins
Types of risk model

- **Absolute Risk Model** – radiation induces cancers over and above the natural incidence.
  - leukemia follows an absolute risk model

- **Relative Risk Model** – radiation increases the natural incidence at all ages proportional to spontaneous background rates (predicts a larger number of induced cancers in old age following radiation)

- **Time-dependent relative risk** – function of dose, age at exposure, time since exposure, gender, etc.
Risk model development

• “Scholarly” committees (conclusions not mandatory):
  – BEIR – National Academy of Sciences Committee on the Biological Effects of Ionizing Radiation

• Committees that make recommendations:
  – ICRP – International Commission on Radiological Protection
  – NCRP – US National Council on Radiation Protection and Measurements (Many of the same people!)
Cancer latency

• Leukemia has the shortest latency of about 5 years

• Whereas, solid cancers have a latency of 20 or more years following radiation
Risk related to initiation upon radiation exposure

A. INITIATION

Excess risk/year due to initiation

Age (years)

Shuryak et. al., JNCI 2010
Risk related to promotion upon radiation exposure
Risk related to initiation and promotion upon radiation exposure

Shuryak et. al., JNCI 2010
Example of myeloid leukemia in male mice given total body x-irradiation
Leukemia in A-bomb survivors
Solid cancers A-bomb survivors
Life-shortening in mice as a function of the dose of ionizing radiation. The shortening of lifespan is ascribed to early death owing to induced cancers.
**Age at onset of XP symptoms.** Age at onset of cutaneous symptoms (generally sun sensitivity or pigmentation) was reported in 430 patients. Age at first skin cancer was reported in 186 patients and is compared with age distribution in 29,757 patients with basal cell carcinoma or squamous cell carcinoma in the United States general population.
Age plays a critical role for cancer risk

The data suggest that children and young adults are much more susceptible to radiation-induced cancer than the older aged populations.

Eric Hall, Ph.D.,
FIGURE 10.8  ● The attributable lifetime risk from a single small dose of radiation at various ages at the time of exposure. Note the dramatic decrease in radiosensitivity with age. The higher risk for the younger age-groups is not expressed until late in life. These estimates are based on a relative risk model and on a dose and dose-rate effectiveness factor (DDREF) of 2. (Adapted from ICRP: Recommendations. Annals of the ICRP Publication 60, Oxford, England, Pergamon Press, 1990.)
Site–Specific Risk Estimates

For person age 70 exposed at age 30

Eric Hall, Ph.D.,
Lowest dose category with significant increase in cancer risk in Atomic-bomb survivors

- Cancer incidence: 5-100 mSv. Mean: 29 mSv (Pierce et al. 2000)

- Cancer mortality: 5-125 mSv. Mean: 34 mSv (Preston et al., 2003)
Summary

- Data suggest linear dose response with no threshold
  Increased risk: 0-100 mSv

- Women have higher risk than men

- Excess risk continues throughout life
Tissue culture model

- Above 100 rads: the transformation frequency may exhibit a quadratic dependence on doses.

- Between 30 and 100 rads: the transformation frequency may not vary with dose.

- Below 30 rads: the transformation frequency may be directly proportional to dose.
Dose-response curves for the induction of neoplastic transformation in mouse cells by x-irradiation. The upper curve is for BALB/3T3 cells; the bottom curve for C3H/10T 1/2 cells.
Enhancers of Radiation-Induced Transformation In Vitro

Agents

Fractionation of dose
UV radiation
Viruses
Estrogen
Asbestos fiber
Hyperthermia (43°–45°C)

High-LET radiation
Chemical carcinogen
Phorbol ester
Insulin
Iron
Ozone
Transformation incidence of irradiated cells
Radiation + promoter

C3H 10T1/2 cells
Occurrence of secondary cancers following radiotherapy

- Current advances in cancer therapy has increased survival of patients
- The occurrence of radiation-induced secondary cancers is serious concern
- Accurate dosing and dosimetry are critical during radiation therapy
Occurrence of secondary cancers following radiotherapy

- Risk of secondary cancers is hard to assess due to lack of proper control

- In prostate and cervix cancer, surgery is an option

- Higher risk of breast cancer in young patients with Hodgkin lymphoma
Breast cancer

- Japanese female survivors of the A-bomb attacks on Hiroshima and Nagasaki

- Female patients in a Nova Scotia sanatorium subjected to multiple fluoroscopies during artificial pneumothorax for pulmonary tuberculosis

- Females treated for postpartum mastitis and other benign conditions
INCIDENCE OF BREAST CANCER/100,000 WY

Atomic Bomb Survivors 1960-1974

BREAST DOSE (rad)
Massachusetts Fluoroscopy

Incidence of Breast Cancer per 100,000 WY vs Breast Dose (rad)
Pooled Thyroid Cancer Studies

Cohort Studies
- A-Bomb Survivors
- Thymus, Rochester
- Tinea Capitis, Israel
- Tonsils, Chicago
- Tonsils, Boston

120,000 people
3,000,000 person years
700 thyroid cancers
Exposure age ≤15

Case-Control Studies
- Cervical Cancer, Intl
- Childhood Cancer, Intl

Ron et al, 1995

Eric Hall, Ph.D.,
Pooled Thyroid Cancer Dose Response by Age at Exposure

$$\text{ERR}_{\text{Gy}} = 7.7; \text{EAR } 10^4 \text{ PYGy} = 4.4$$

![Graph showing relative risk vs. dose, with two lines indicating different age at exposure groups: one for < 15 and another for >= 15.]

Ron et al, 1995
Pooled Thyroid Cancer Excess Relative Risk

ERR/Gy

Time Since Exposure

Ron et al, 1995

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Second Cancers After Prostate RT

% contribution to total number of radiation-induced second cancers (5+ yrs)

- Bladder [37%]
- Lung [34%]
- Rectum [12%]
- Colon [9%]
- Sarcoma (in field) [6%]
- Sarcoma (out of field) [2%]
## Risk of *Radiation-Associated* Second Malignancy After Prostate-Cancer Radiotherapy

<table>
<thead>
<tr>
<th>All survivors</th>
<th>1 in 290</th>
</tr>
</thead>
<tbody>
<tr>
<td>5+ yrs survivors</td>
<td>1 in 125</td>
</tr>
<tr>
<td>10+ yrs survivors</td>
<td>1 in 70</td>
</tr>
</tbody>
</table>

*Brenner et al 1999*

Eric Hall, Ph.D.
Lung Cancer after Hodgkin’s Disease by Type of Treatment *

- **Alkylating agents only**: RR=4.2, 73 Ca, 135 Co
- **Radiotherapy (RT) only**: RR=5.9, 21 Ca, 98 Co
- **RT and alkylating agents**: RR=8.0, 52 Ca, 70 Co

*Adjusted for tobacco use*

*Travis LB, et al. JNCI, 2002*
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