Radiation Carcinogenesis

November 22, 2016

Dhyan Chandra, Ph.D.
Pharmacology and Therapeutics
Roswell Park Cancer Institute
Email: dhyan.chandra@roswellpark.org
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- Henri Becquerel discovered radioactivity
- 1897- Rutherford discovered $\alpha$ and $\beta$ rays
- 1898- Curies discovered polonium and radium
- 1902- First report on radiation-induced skin 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:
- α particles (2 protons and 2 neutrons)
- β particles (electron equivalent)
- Neutrons
- Gamma rays
- X-rays

Non-ionizing radiation:
- Microwaves
- Visible light
- Radio waves and TV waves
- UV radiation (except shortest wavelengths)
Gamma Rays and EM Spectrum

Electromagnetic radiation with wavelength of $\sim 10^{-12} \text{ m}$. 

Approximate Scale of Wavelength

- Buildings
- Humans
- Butterflies
- Needle Point
- Protozoans
- Molecules
- Atoms
- Atomic Nuclei

Frequency (Hz)

- $10^4$
- $10^8$
- $10^{12}$
- $10^{15}$
- $10^{16}$
- $10^{18}$
- $10^{20}$
Ultraviolet / Visible Light Spectrum

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.
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 (roentgen equivalent in man) - Old unit of equivalent or effective dose. It is the product of absorbed dose (in rad) and the radiation weighting factor. 1 rem = 0.01 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 γ-ray:  x  x  x  Sparsely Ionizing

β particle:  x  x  x  x

Neutron:  x  x  x  x  x  x  x  Densely Ionizing

α particle  xxxxxxxxx

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 (use of thorium dioxide as radiocontrast agent in medical radiography in 30s-40s)
Later cases of human experience

- Hiroshima/Nagasaki survivors
- Radiation treatment of Anklyosing Spondylitis patients (arthritis of spine)
- 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 by radiation
- Breast cancer due to frequent chest X-Ray fluoroscopy in tuberculosis patients between 1925 to 1954
Sources and consequences of DNA damage

- **Exogenous Sources**
  - UV and other radiation sources, chemicals

- **Endogenous Sources**
  - ROS, alkylation, hydrolysis

- *Single-strand break*

- **DNA damage**

  - Misreplication, aberrant chromosomal segregation
    - Mutations, chromosomal aberrations
      - Cancer

  - Blocked transcription
    - Blocked replication
      - Cell-cycle delay or arrest, cell death
      - Aging

**DNA repair systems**
Measurements of DNA damage

DNA in Cells

No Radiation  + Radiation

Isolated Lesions  Clustered Damages

SSB  OxyBase  DSB  OxyBase Cluster

Glycosylase/Lyase

-  +  -  +

SSB  Oxybase  DSB  Clusters

Neutral Agarose Gel

ML  1  2  3  4  ML
Measurements of DNA damage

(a) Control and irradiated samples at different time points.

(b) Number of γ-H2AX foci over time post-irradiation.

(c) DNA damage (OTM) over time post-irradiation.

(d) % DNA DSBs over time post-irradiation with different doses.

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
Major regulatory steps in the process of DNA damage response

- **γ-H2AX**
  - DNA-PK complex
  - (RF-C)-like complex
  - RAD54
  - RAD52
  - 53BP1
  - BRCA1
  - BRCA2

- **DNA damage sensors**
  - NBS1
  - RAD50
  - MRE11
  - PCNA-like complex

- **Upstream kinases**
  - ATM
  - ATR

- **DNA damage mediators**
  - ATRIP
  - MDC1
  - 53BP1
  - CHK1

- **Downstream transducer kinases**
  - CDC25
  - E2F
  - p53

- **DNA damage effectors**
  - PML
  - SMC1
DNA damage and human cancer

EXOGENOUS SOURCES
IR: X, Y rays, α-particles
UVA radiations, chemicals and drugs

ENDOGENOUS SOURCES
O₂ metabolism, immune response inflammation

Intracellular radical scavengers
Antioxidant enzymes
Vitamins C, E

ROS/RNS

Bystander cell
Chemokines
Cytokines

Isolated DNA lesions
SSB
5’ Oxidized base 3’
3’ Oxidized base 5’

Oxidative Clustered DNA lesions (OCDLs)

Repair by BER and/or NER
DNA replication
Mutations
Genomic instability
Cancer cell

Pre-cancerous state
Persistent DNA damage

DNA Damage repaired
Cell death
E2F targets are shown in red

p53 targets are shown in blue

Targets for E2F and p53-purple
DNA damage, autophagy, and Cancer

http://dx.doi.org/10.1016/j.redox.2014.12.003
Radiation-induced chromosomal aberrations

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

1) Rejoin to original configurations
2) The breaks fail 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

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.

Prise and Sullivan 2009
Cancer incidence at various ages

[Graph showing cancer incidence rates per 100,000 people for men and women across different ages, with separate lines for prostate, colon/rectum, lung/bronchus, stomach, urinary bladder, and pancreas for men, and breast, colon/rectum, lung/bronchus, pancreas, urinary bladder, ovary, and uterus for women.]

Figure 11.1 The Biology of Cancer (© Garland Science 2014)
Multistep tumorigenesis in variety of organ sites

Colon
- Normal
- 5–20 years → Initiated
- Adenoma
- 5–15 years → Pre-cancer
- Cancer

Head and neck
- Normal
- Tobacco use 4–10 years → Initiated
- Dysplastic oral leukoplakia
- 6–8 years → Pre-cancer
- Cancer

Cervix
- Normal
- CIN 1
- 9–13 years → Pre-cancer
- CIN 3/CIS
- 10–20 years → Pre-cancer
- Cancer

Lung (smokers)
- Normal
- Atypical hyperplasia
- 20–40 pack-years → Pre-cancer
- DCIS
- 6–10 years → Pre-cancer
- Cancer

Breast
- Normal
- Atypical hyperplasia
- 20 years → Pre-cancer
- PIN
- ≥10 years → Pre-cancer
- Latent cancer
- 3–15 years → Pre-cancer
- Cancer

Figure 11.8a The Biology of Cancer (© Garland Science 2014)
Mouse skin model

**Initiation**
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.

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

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

**Genetic Susceptibility**
1. Additional genetic events occurring stochastically.
2. Aneuploidy e.g., nonrandom trisomies of chromosomes 6 & 7.
3. LOH.
4. Further alteration in differentiation.
5. Invasion
7. Loss of tumor suppressor activity e.g., p53 mutation.

**Other**
1. Invasion
Overview of carcinogenesis

- External Exposure
  - Metabolic Activation
    - DNA Damage
      - Fixation
        - Initiation
        - Promotion
        - Progression
          - Preclinical Biologic Effect
          - Cancer
            - Latency Period

- Internal Exposure
  - Biologically Effective Dose
    - DNA Repair
      - Excretion
        - Detoxification
          - Excretion
            - Exposure
              ~ 1 day

- Effects
  ~ 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
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 differentiate.
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.
UVB

Skin

Photochemopreventive Agents Act As

- Sunscreen (1st line)
  - Prevention of Damage
  - Gene inactivation

- Antioxidant (2nd line)
  - Radical scavenger
  - Oxidative stress
  - DNA damage
  - DNA excision repair
  - p53

- Redox regulation of signal transduction pathway (3rd line)
  - Inflammation
  - Proliferation
  - Transformation specific apoptosis in transformed cells
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.
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
Risk related to promotion upon radiation exposure

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

Example of myeloid leukemia in male mice given total body x-irradiation
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.
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

ERR, Excess Relative Risk

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.
Transformation incidence of irradiated cells
Radiation + promoter

C3H 10T1/2 cells

Graph showing transformation frequency per viable cell vs. dose (rads) for IR and IR+TPA treatments.
Occurrence of secondary cancers following radiotherapy

- Current advances in cancer therapy has increased survival of patients

- The occurrence of radiation-induced secondary cancers is a 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
Second Cancers After Prostate RT

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

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

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

*Source: Brenner et al 1999*

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

P trend <0.001

Relative Risk*

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

Travis LB, et al. JNCI, 2002
*Adjusted for tobacco use

Eric Hall, Ph.D.
Summary

- 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