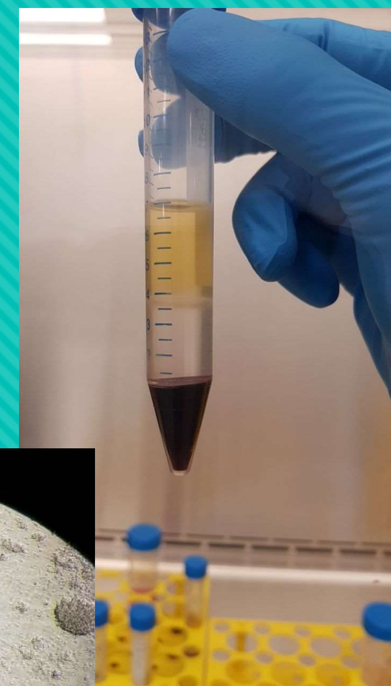
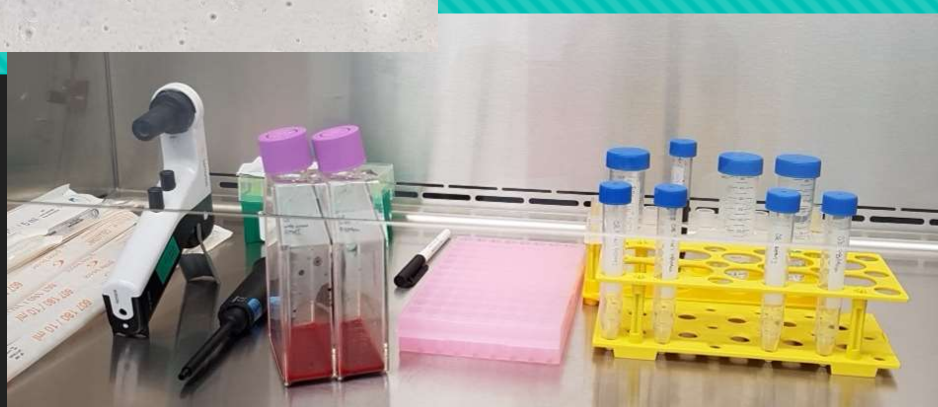
Biological Effects of Ionising Radiation

Dr Christelle Chua

Singapore Nuclear Research and Safety Initiative

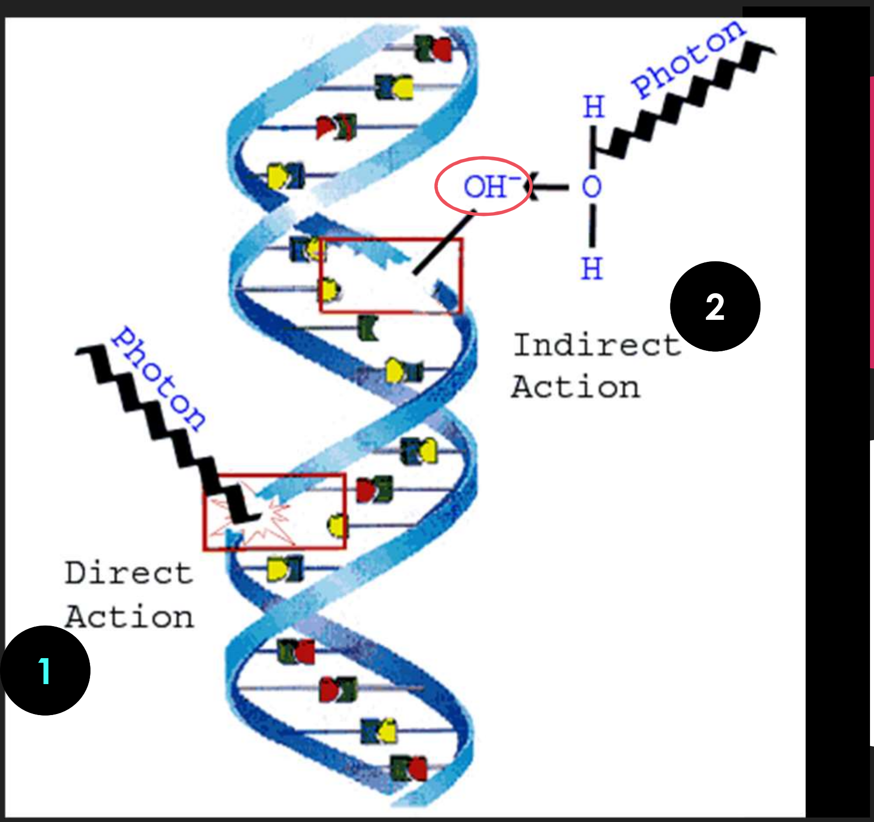
snrccel@nus.edu.sg





Contents

Molecular effects: Direct and indirect action



Direct action: Radiation hits molecule directly, disrupting molecular structure due to high energy (1)

Indirect action: Radiation hits water (and other) molecules, resulting in free radicals which have unpaired electrons (2)

Free radicals are highly reactive with other molecules, resulting in damage

https://www.radtrainonline.com/courses/slideimages/DNAstrand11.gif

Cellular effects: responses to DNA damage

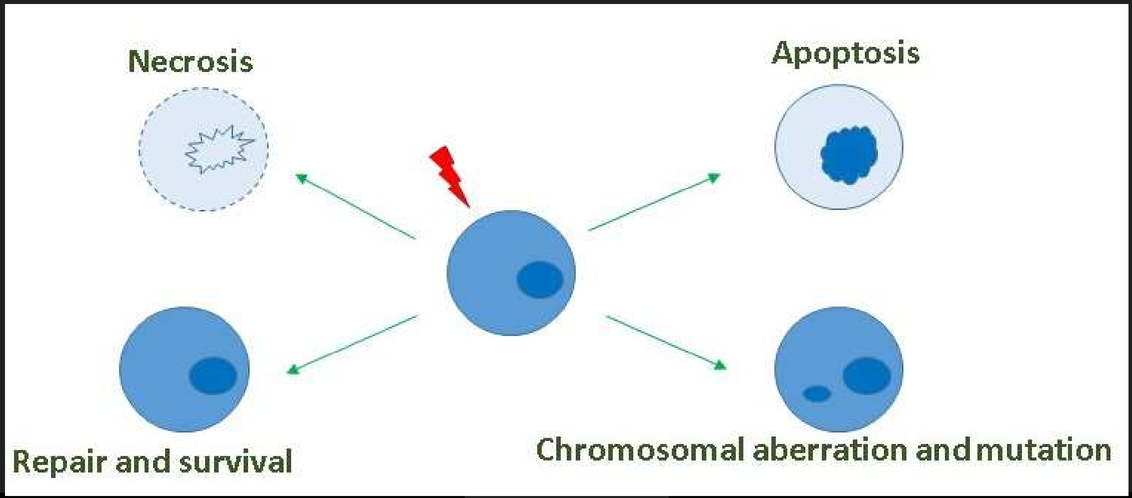
NECROSIS

► Accidental or programmed cell death resulting from loss of membrane integrity

- May give rise to inflammation

APOPTOSIS

► Programmed cell death



REPAIR & SURVIVAL

► Cells can attempt to repair DNA damage

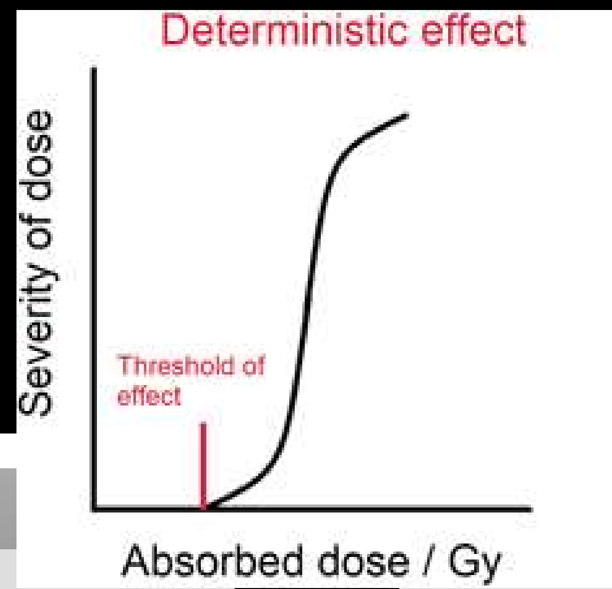
MUTATION

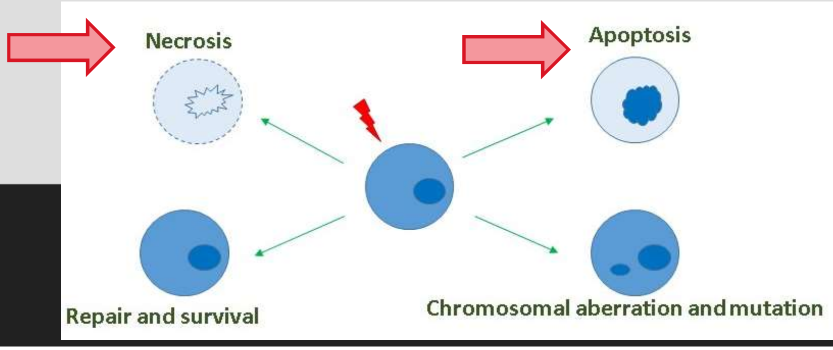
► DNA damage is incorrectly repaired but cells continue to survive

- Mutations passed on to progeny

Physiological effects: Deterministic effects

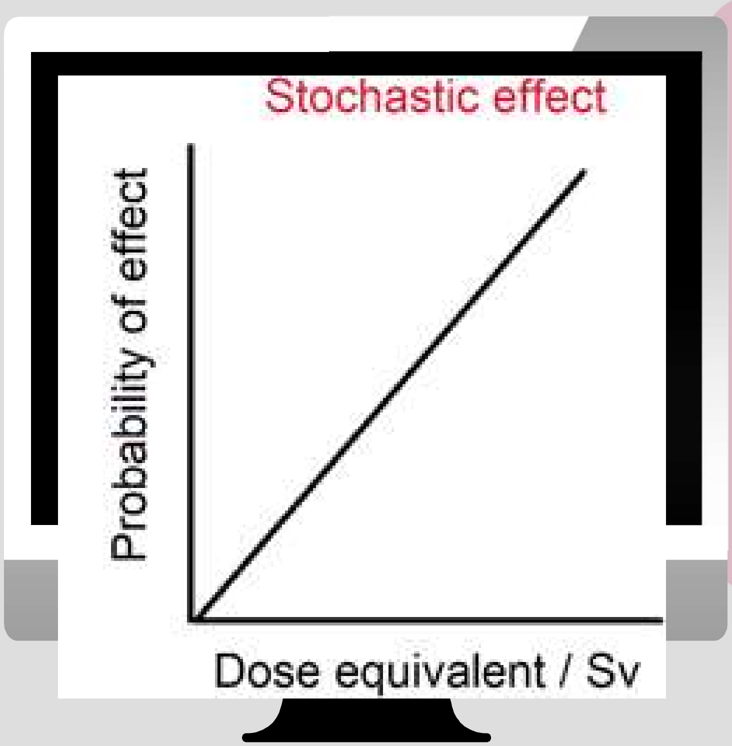
- Severity increases as the dose increases
- Only occur once a threshold of exposure has been exceeded
- Usually noticeable soon after exposure
- Due to large-scale cell death causing functional impairment of a tissue or organ

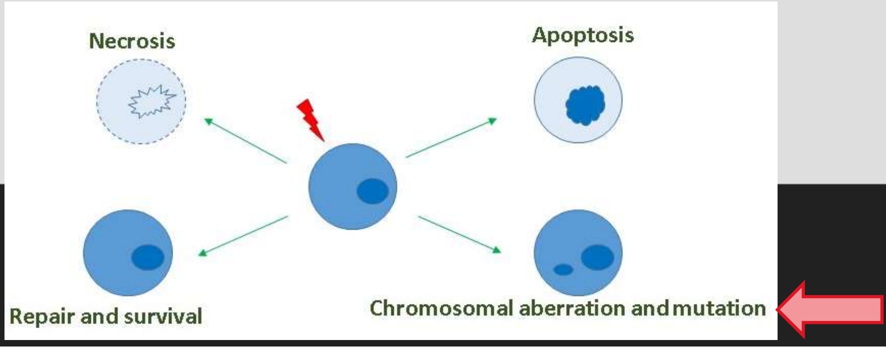




Physiological effects: Stochastic effects

- Risk (probability) increases linearly with dose
- No threshold level for effects and severity is not dose related
- Usually occurs at later time e.g. cancer / hereditary diseases
- Generally due to accumulation of mutations





Factors affecting the extent of biological response to radiation

- Type of ionising radiation
- Type of tissue exposed
- Dose
- Dose rate
- Route of exposure
- Individual health

Contents

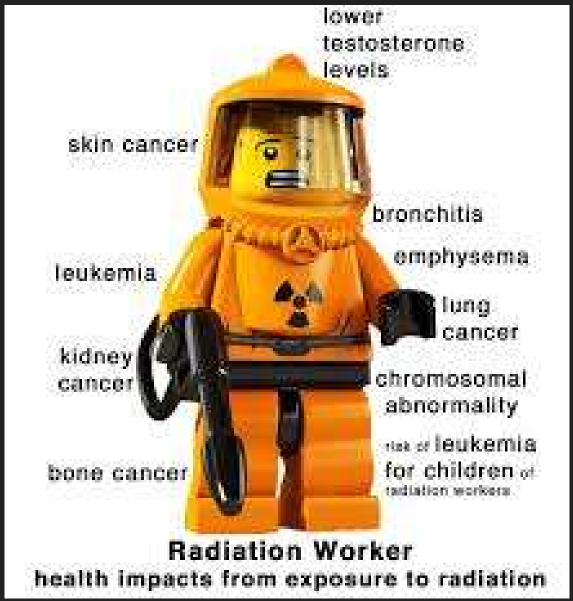
1. Molecular, cellular and physiological effects
2. Factors that impact the extent of biological effects
3. Methods of studying biological effects of radiation exposure
4. Applications and implications

Varied conclusions on risk associated with chronic exposure

	Exposure	Negative effects	No negative effects
Epidemiological studies	Patients (X-rays)	• Halm et al., (2013) – genotoxic effects	• Mortazavi et al., (2003) – adaptive response
	Nuclear workers (gamma sources)	• Sahin et al., (2009) – genotoxic effects • Picano et al., (2012) – non-cancer effects • Leuraud et al., (2015) – leukemia • Howe et al., (2004) – cancer and non-cancer effects	• Fornalski et al., (2010) – hormesis effect
	Background radiation	• Hwang et al., (2008) – cancer	• Thompson et al., (2008) – negative relative risk for cancer
	Areas of nuclear accidents	• Preston et al., (2007) – cancer • Kamiya et al., (2015) – non cancer effects	• Kamiya et al., (2015) – no difference in cancer risk
Animal studies	Iodine-125		• Olipitz et al., (2012) – DNA damage seen in acute exposure but not in CLD or control
	Cs-137	• Kempf et al., (2016) – contributing risk factor for Alzheimer's disease	• Osipov et al., (2007) – activation of defense system in spleen cells
	X-ray	• Goudarzi et al., (2014) – Profile of urine in control vs CLD vs high acute are different	
Ex vivo studies	Cs-137	• Loseva et al. (2014) – senescence induced in fibroblasts • Katsura et al. (2016) – altered gene expression in neural progenitor cells	
	X-ray	• Ghandhi et al. (2015) – acute exposure triggers more mechanisms involved in metabolism while CLD triggers more mechanisms involved in DNA repair	

Studying the effects of exposure by epidemiology

1. **Epidemiological studies**: study of human populations to link disease with a cause
   - Populations of interest:



http://ep-let.gek.szie.hu/sites/default/files/2017_III_Kornyezetgazdalkodas.pdf

Epidemiological study of people from Chernobyl

- From the 2000 UNSCEAR* report

Type	No. of ppl	Dose received (Gy)
First responders	600	2-30
Recovery operation workers	600 000	0.1
Evacuees	100 000	Thyroid: 0.3-1; External: 0.03
Residents of contaminated areas	6 000 000	Thyroid: 0.2; External: 0.01

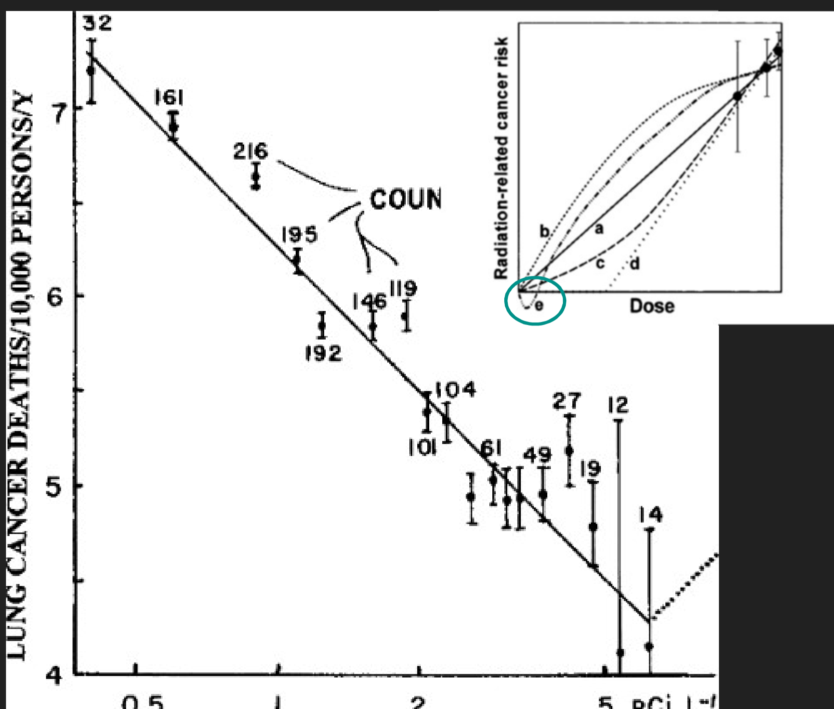
- 47 deaths from 1986 to the year 2004
- More than 4,000 developed thyroid cancer (1992 to 2002), of which less than one per cent died
- "apart from this (thyroid cancer) increase, there is no evidence of major public health impact attributable to radiation exposure 14 years after the accident. There is no scientific evidence of increases in overall cancer incidence or mortality or in non-malignant disorders that could be related to radiation exposure".

*UN Scientific Committee on the Effect of Atomic Radiation

http://www.irpa.net/irpa10/cdrom/00713.pdf

Epidemiological study of radon exposure

Data on lung cancer mortality rates vs. average radon concentration in homes for 1,601 U.S. counties

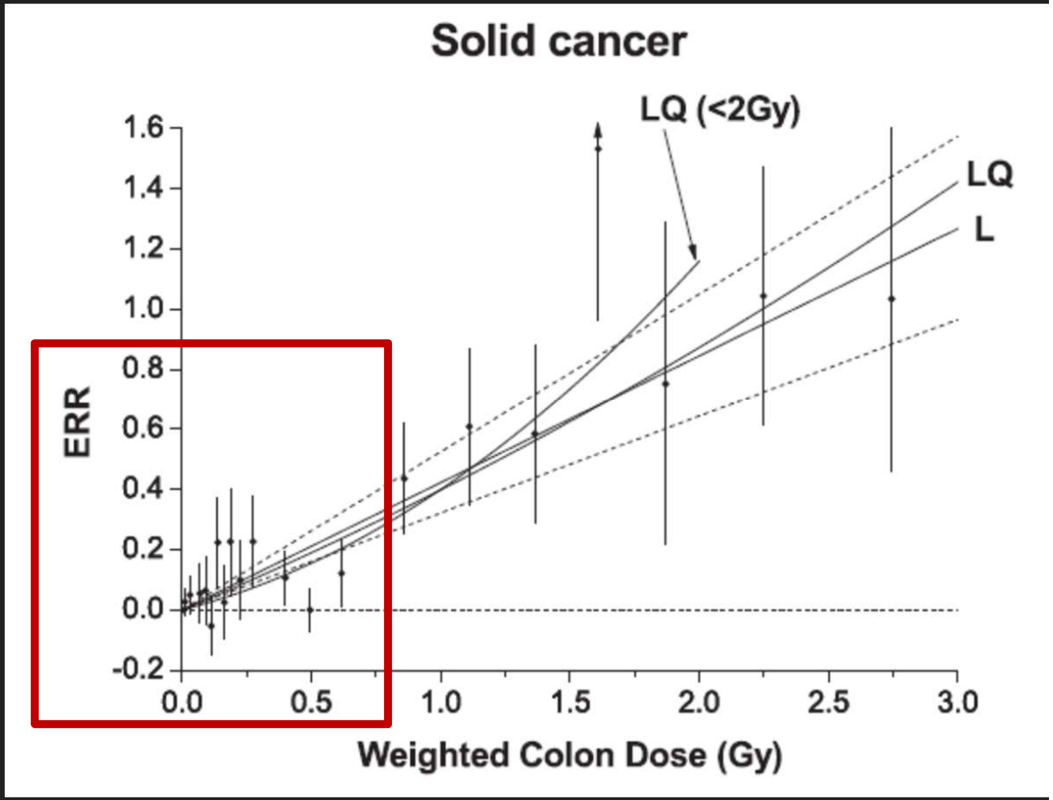


- Took into account confounding factors including socioeconomic factors
- At low doses, hormesis model appeared to fit the data: "failure of the linear-no threshold theory for carcinogenesis from inhaled radon"
- Note: Some researchers argue that the authors did not appropriately take into account confounding factors such as smoking and lifestyle

Cohen. (1995) *Health Phys.* 68:157-74.

Epidemiological study of survivors of atomic bomb

- Mortality study since 1950 on a fixed population [Life Span Study (LSS) cohort*] of about 120,000 subjects including atomic bomb survivors and residents of Hiroshima and Nagasaki
- Dose estimates for survivors within 2 km are based on histories obtained through interviews
- Trend at lower doses remain unclear



Ozasa et al., (2012) *Radiat Res* 177:229-243

**Conducted by Radiation Effects Research Foundation, Japan*

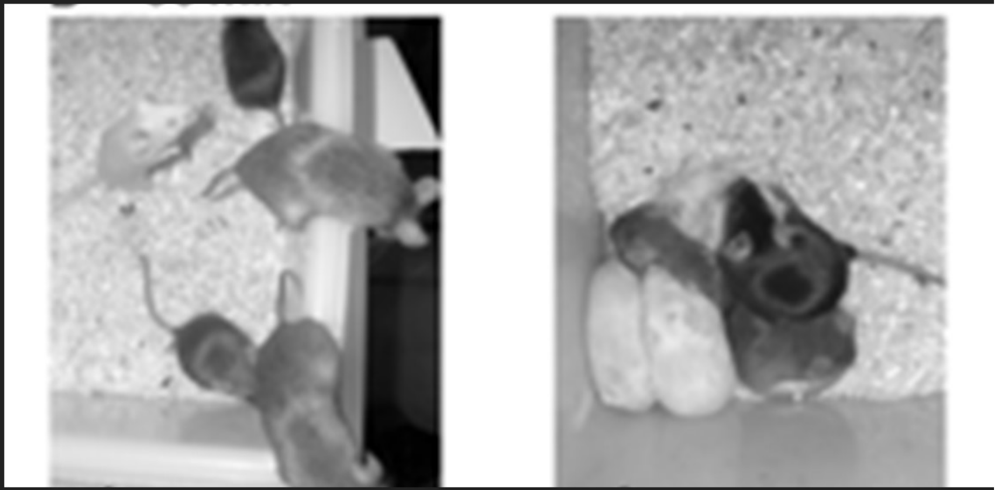
Studying the effects of exposure with animal studies

2. **Animal studies**: use of animal models to study the effects after deliberate exposure to radiation
   - Aspects of study:

http://www.sciencemag.org/news/2017/07/memory-enhancing-drug-reverses-effects-traumatic-brain-injury-mice

Behavioural changes observed in animal studies

- Neonatal male mice irradiated with 200mGy gamma irradiation from Co-60 source daily from post-natal day 10-12
- At 2 months, mice were tested for exploratory behavior in a novel home environment and subsequent habituation
  - reveals ability to integrate new information
- Irradiated mice had reduced exploratory behavior and habituation, suggesting developmental deficits



https://academic.oup.com/hmg/article/14/16/2369/675665

Buratovic et al., (2016) *Int J Radiat Biol* 92:371-379

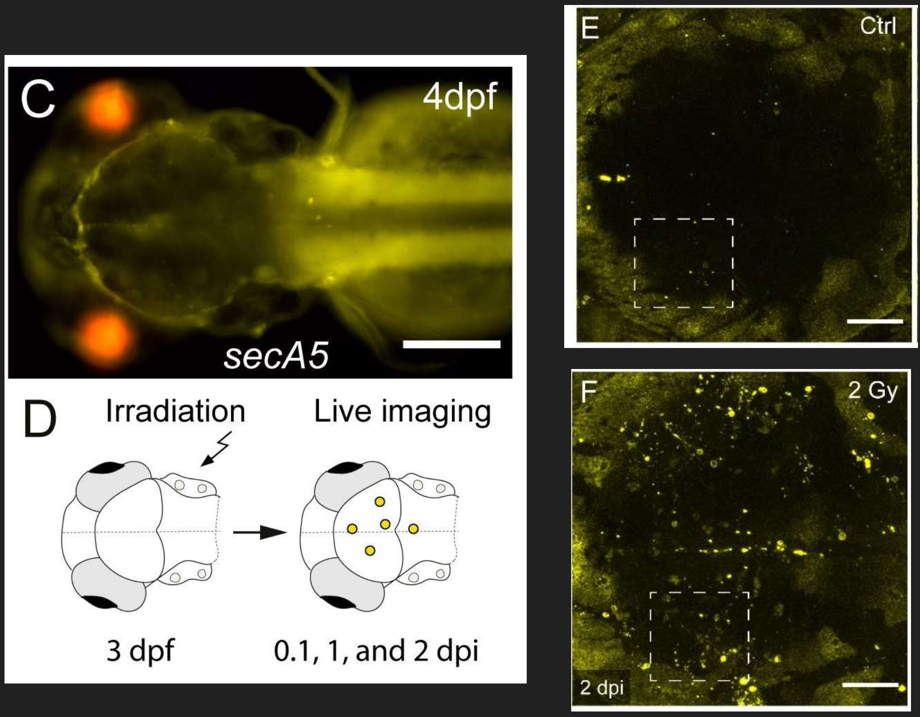
Example of molecular and physiological changes in mice

- Mice were chronically irradiated with gamma source for 9 months at 12 and 28 μGy/h
- These mice are genetically modified to reflect the disease atherosclerosis (buildup of plaque in arteries)
- Researchers found a decrease in plaque size in exposed mice compared to non-exposed mice
- Anti-inflammatory and anti-oxidative gene expression was also observed
- Hormesis effect?

Ebrahimian et al. (2018) *Radiat Res* 189:187-196

Zebrafish as a model to study cellular effects

- Zebrafish as an example of another animal model that can be used to study effects of radiation
- Zebrafish embryo are transparent and can be easily imaged with microscopy
- Transgenic zebrafish with a fluorescent reporter for apoptosis were irradiated at 2 Gy



Sun LWH, Asana Marican HT, Shen HY. (2023) *Radiat Res* 2023 (accepted)

Studying the effects of exposure ex *vivo*

3. ***Ex vivo* studies** on human cells/tissue: irradiation of cells/tissue obtained from human donors to study the effects
   - Bioindicators of interest (characteristic that is objectively measured and evaluated as an indicator of biologic processes):
     - Chromosomal aberrations
     - Apoptosis
     - Changes in RNA and protein levels

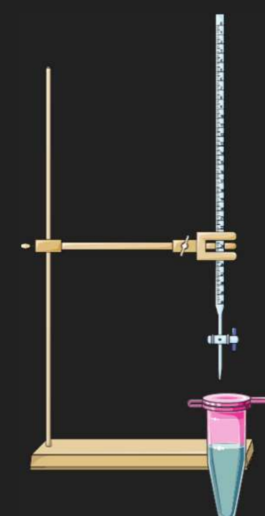
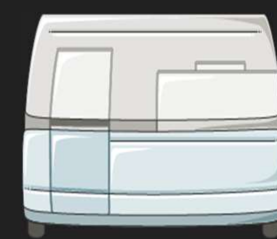
Primary cell culture: *ex vivo* culture of cells obtained directly from an organism

Continuous cell lines: Transformed for be able to divide indefinitely

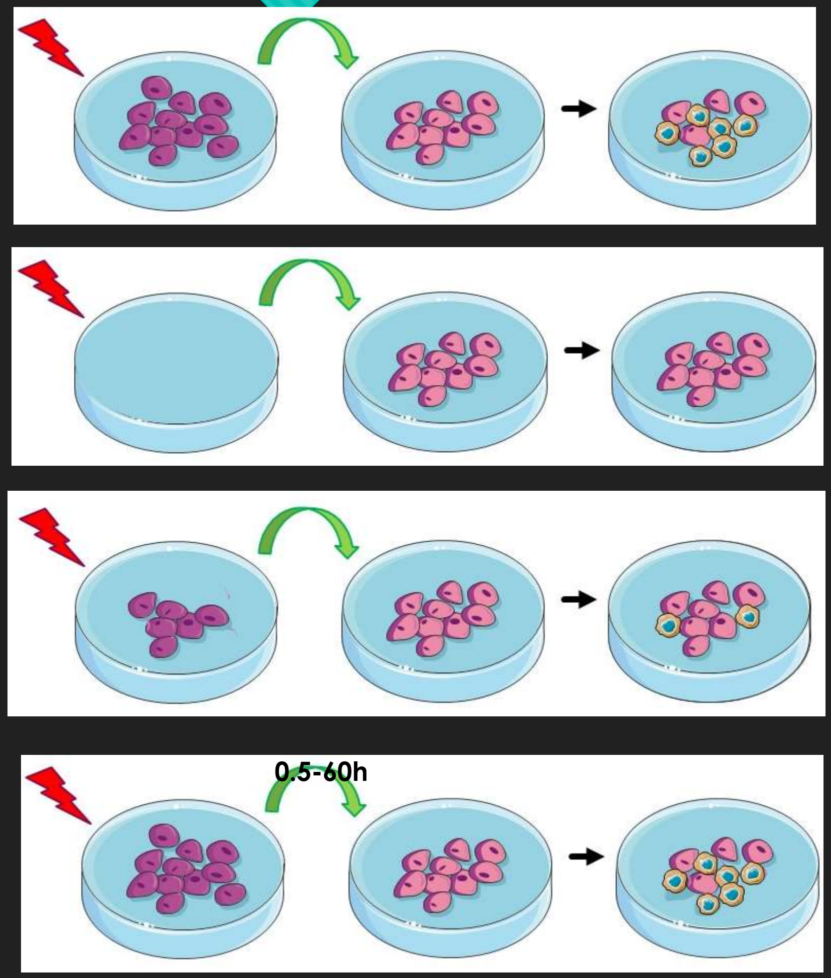
General protocol for ex *vivo* studies

Sample withdrawal from human donors → Ex vivo culture → Exposure to irradiation → Extraction of bioindicator → Quantification of bioindicator



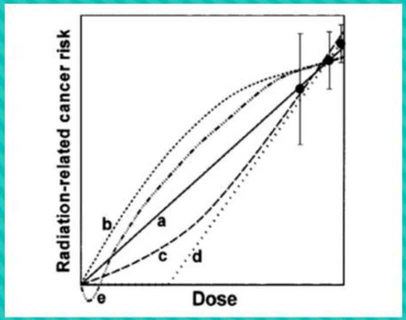
Radiation induced bystander effect observed by ex *vivo* studies



- Something present in culture media mediates radiation induced bystander effect (RIBE)
- RIBE is not due to generation of ROS from components in the culture medium alone
- RIBE is mediated by some component released from irradiated cells into the culture media
- This component is not likely to be ROS alone, but something more long-lived

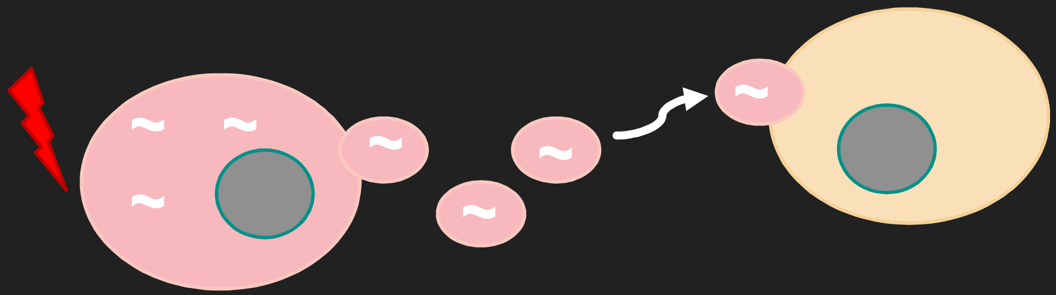
Mothersill et al., (1997) *Int J Radiat Biol* 71:421-427

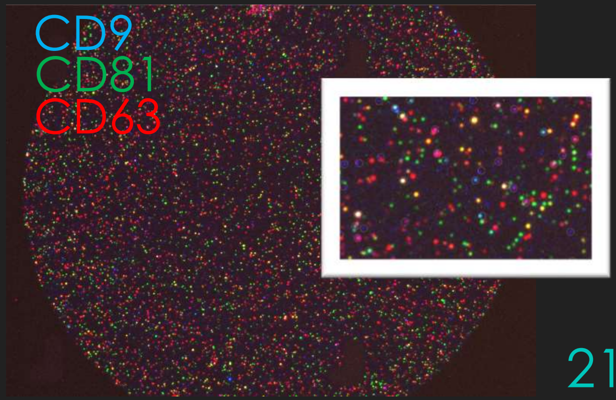
Mediators of RIBE



- Role of cytokines
  - Cytokines: small proteins involved in inter-cell communication e.g. TNF-a
  - Introduction of antibody that inhibits TNF-a reduces RIBE

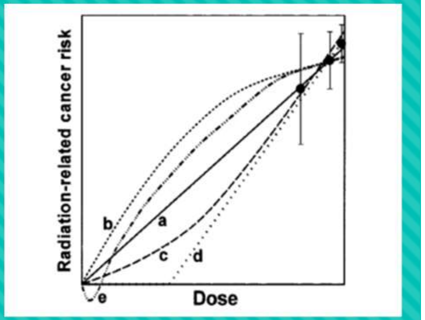
- Role of exosomes
  - Exosomes: tiny vesicles (fluid-filled sac) released from a cell
  - Exosomes isolated from conditioned media can mediate RIBE



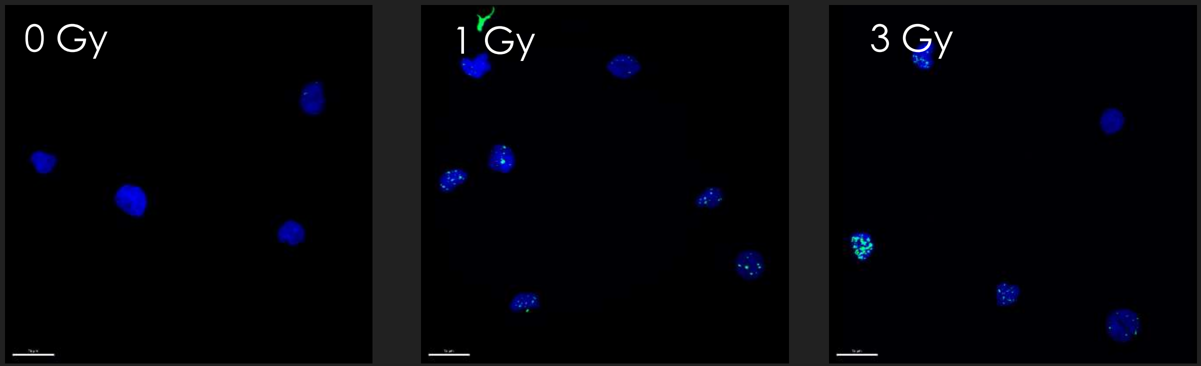


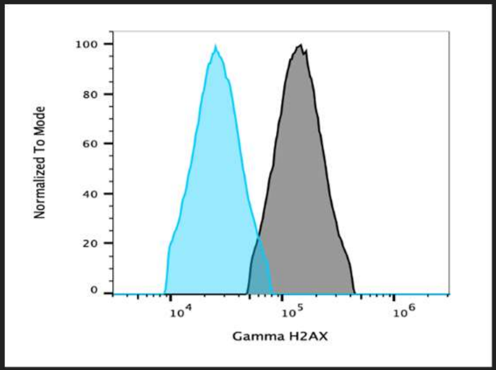
Zhou et al., (2008) *Cancer Res* 68:2233-2240
Xu et al., (2015) *RNA Biol* 12: 1355-1363

Adaptive effects observed by ex *vivo* studies



- Cells treated with a chronic, low dose exposure followed by a challenge with a high dose exposure
- Observe amount of DNA damage by looking at presence of γ-H2AX foci (a protein marker which gathers at sites of DNA damage)





Cells with prior low dose exposure have reduced sites of DNA damage

More complex ex *vivo* models have been developed

- While traditional *ex vivo* cell cultures have been conducted in 2D (attached to culture flasks or in suspension), newer methods have allowed for cells to be cultured in a state that is more similar to physiological systems
- 3D culture systems:
  - Cells can be grown on scaffolds that mimic the extracellular environment
  - Organoids (mass of cells that can mimic complex organ functions)
  - Microfluidics can mimic gas and fluid exchange

Why the large variation in results from different research?

- Different model systems used
  - Animals and humans have different susceptibility
- Different types of radiation and dose / dose rates
- Different end points being studied
  - Cancer is a multi-factorial disease
- Different length of study
  - Stochastic effects may take a long time to develop

What are the merits of the various methods?

	Advantage	Disadvantage
Epidemiological		
Animal		
Ex vivo		

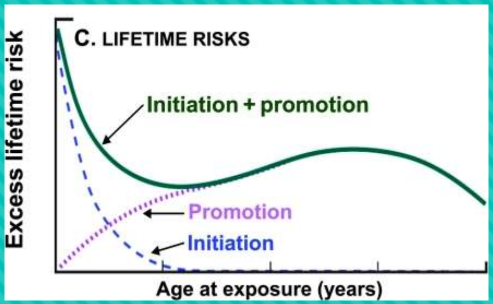
Contents

1. Molecular, cellular and physiological effects
2. Factors that impact the extent of biological effects
3. Methods of studying biological effects of radiation exposure
4. Applications and implications

Why study biological effects of radiation?

- **Influence on policies**
- Use in biodosimetry (assessment of radiation dose)
- Impact on ecology
- Implications in medicine

The concept of risk



- **Absolute risk** is defined as the probability that a person who is disease free at a specific age will develop the disease at a later time following exposure to a risk factor
- **Relative risk** model assumes radiation increases the natural incidence of a cancer and it is expressed as a fraction or multiple of the naturally occurring risk

Table 2: Nominal Risk for Cancer Effects*	
Exposed Population	**Excess Relative Risk of Cancer (per Sv)**
entire population	5.5% – 6.0%
adult only	4.1% – 4.8%
**relative risk values based on ICRP publications 103 (2007) and 60 (1990)*	

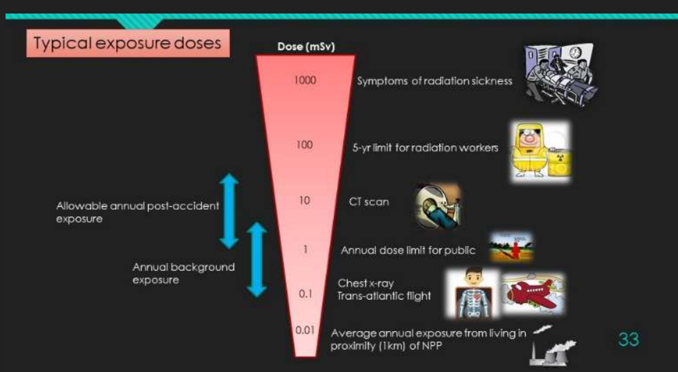
*International Commission on Radiological Protection: independent international non-governmental organization to provide guidance on radiation protection

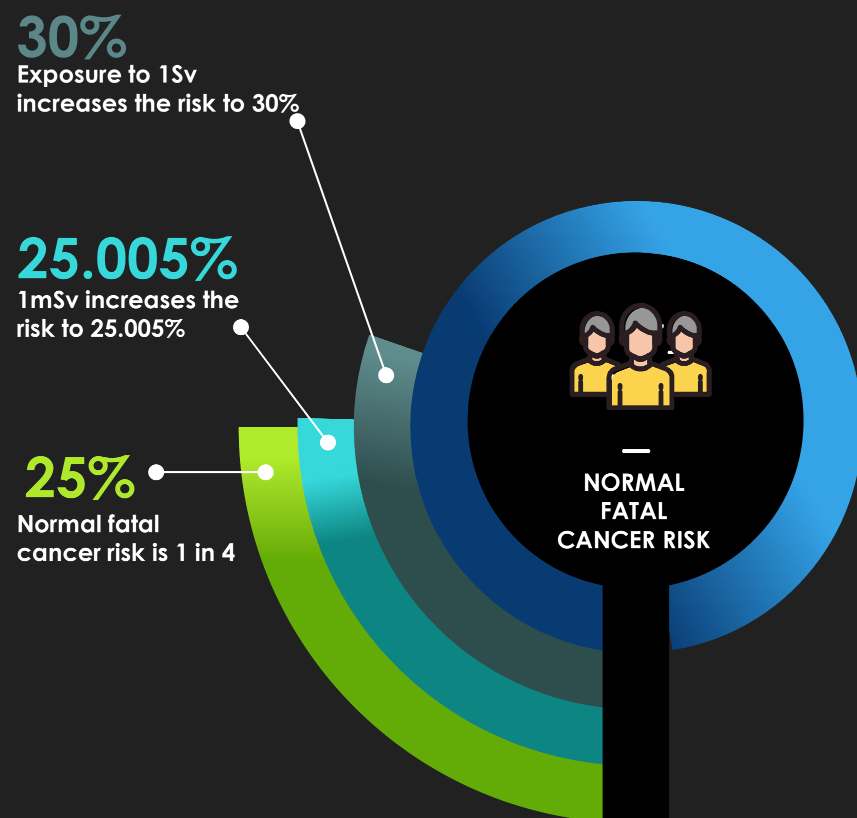
Putting risk into perspective

5% / Sv Fatal Cancer Risk

=0.005% per mSv

If 20000 persons were exposed to 1 mSv, one person will develop a fatal cancer





http://www.shsccentre.com/2017/06/20/radiation-concerns/

What is considered risky?

- For 10, 000 persons receiving 10 mSv of radiation, an additional 8 people will be at risk of cancer death

Activities with similar risk

Driving 40 miles by car

Smoking 1.4 cigarettes

Eating 40 spoons of peanut butter

http://www.shsccentre.com/2017/06/20/radiation-concerns/

Ultimately, risk is a matter of perception and choice

Policies on use of radiation in medicine

- There appears to be an associated risk of cancer in patients that receive CT scans
- Authors suggest reducing CT doses and frequency of usage as a medical tool, especially on young, otherwise healthy individuals

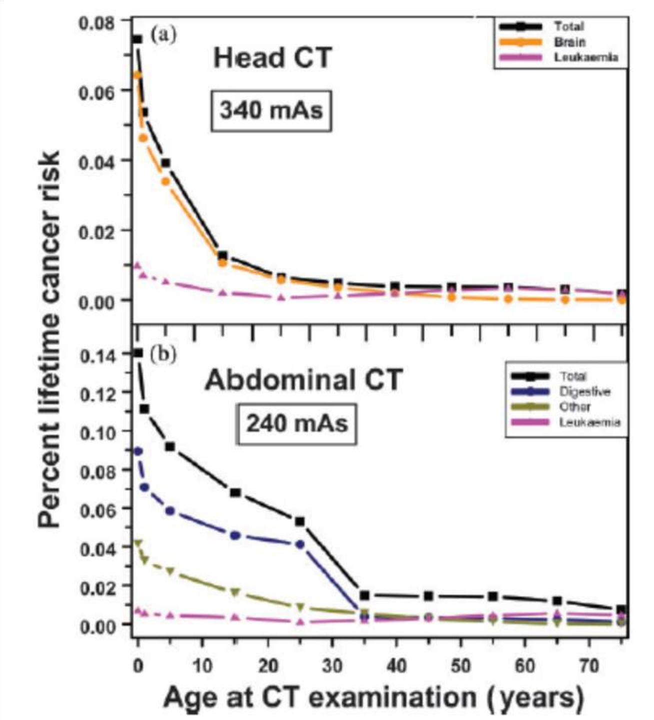


Figure 11. Estimated age-dependent, gender-averaged percentage lifetime radiation-attributable cancer risks from typical single CT scans of (a) the head and (b) the abdomen [3], based on estimated organ doses shown in Figure 3. The methodology used is summarized in the text. The risks are highly age dependent, both because the doses are age dependent (Figure 3) and because the risks per unit dose are age dependent (Figure 6). Despite the fact that doses are higher for head scans, the risks are higher for abdominal scans, because the digestive organs are more sensitive to radiation-induced cancer than is the brain.

Recall some concepts that affect radiobiological response?

Hall et al., (2008) *British J Radiol* 81:362-378

Policies on safe levels of low level exposure

- "Perhaps one-third of the radon-attributed cases (about 4% of the total lung-cancer deaths) would be avoided if all homes had concentrations below the Environmental Protection Agency's action guideline of 150 Bqm^{-3} (4 $pCiL^{-1}$);"

VS

- "The importance of this use of the LNT is difficult to exaggerate. It is estimated that in the USA, US$85 billion will be spent in cleaning up the Hanford site* to avoid low level radiation (LLR)... If the LNT is wrong and LLR is harmless, all of this money will be wasted"

*Decomissioned and demolished nuclear production complex in US

BEIR VI Committee . Health Effects of Exposure to radon.Washington, DC: The National Academy Press; 1999
Cohen (1998) *23rd Ann Symposium Uranium Insitute* 149-166

Policies on compensation and liability


THE STRAITS TIMES ASIA LOG IN ST SUBSCRIBE

Six sue Fukushima nuclear plant operator over thyroid cancer

PUBLISHED
JAN 27, 2022, 11:12 AM SGT

"No causal relationship between radiation exposure from the disaster and thyroid cancer has been recognised by an expert panel set up by the regional government.

A higher rate of thyroid cancer detected among children exposed to the radiation was likely due to better diagnostics, the UN's Scientific Committee on the Effects of Atomic Radiation concluded.

But the plaintiffs' lawyers say none of the cancers suffered by the group were hereditary, arguing it is highly likely the disease was caused by exposure to radiation"

Why study biological effects of radiation?

- Influence on policies
- **Use in biodosimetry (assessment of radiation dose)**
- Impact on ecology
- Implications in medicine

Biodosimetry

- A clinical observation or laboratory result that correlates with the approximate radiation dose estimated to have produced that effect

- Aim is to
  - determine the dose received
  - aid in the decisions required for countermeasures
  - assess the risk of long term consequence of exposure

- This may be important in scenarios such as mass casualties in radiation emergencies, as victims are not likely to be wearing dosimeters and results from area surveys may take a long time

Adapted from Radiation Dose Reconstruction: Principles and Practices, NCRP Report No. 163, page 182, Bethesda, MD, 2009

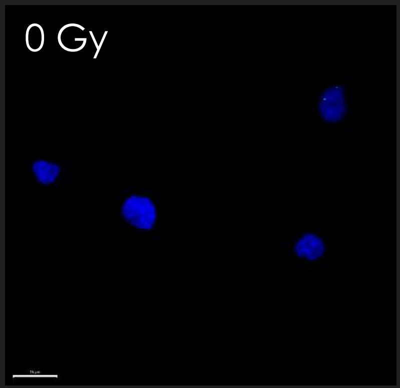
Biodosimetric markers

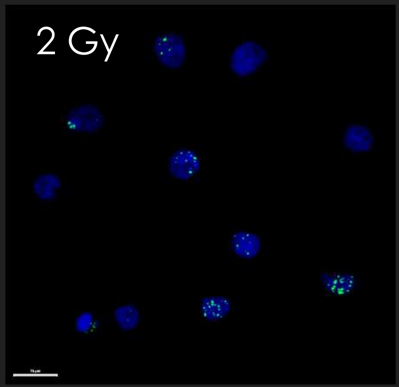
- Can be physiological, chemical or biological markers in human tissues after exposure to ionizing radiation

- E.g.
  - DNA damage markers
  - Chromosomal aberrations
  - Changes in mRNA and protein levels
  - Appearance of phenomena associated with ROS

DNA damage foci

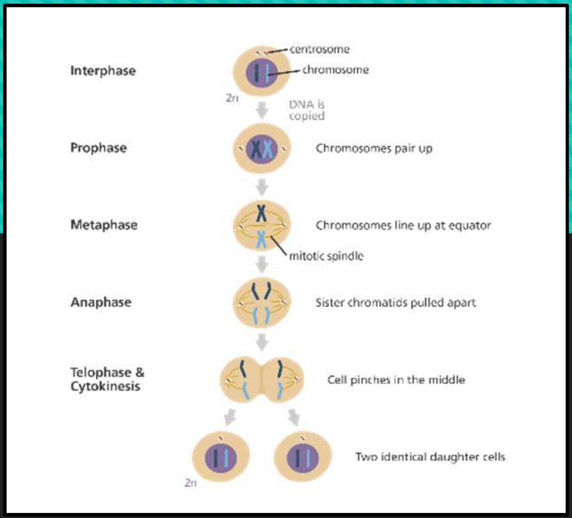
- DNA double strand breaks trigger a cellular response, including the recruitment of the phosphorylated protein H2AX (γH2AX) as part of the DNA repair process
- Accumulation of γH2AX can be visualised as discrete foci with fluorescent labelling and microscopy
- Can be correlated to the amount of DNA damage



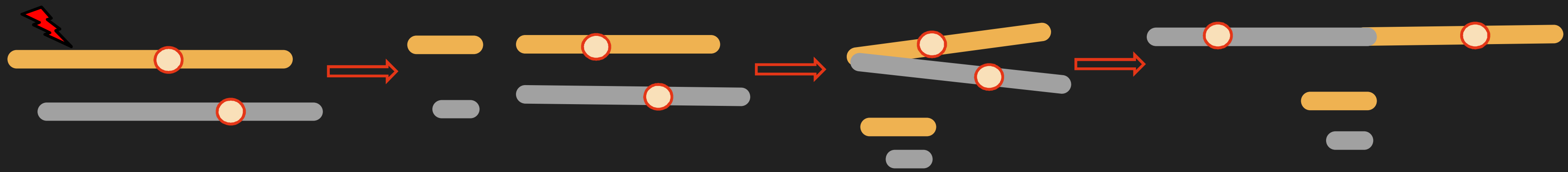


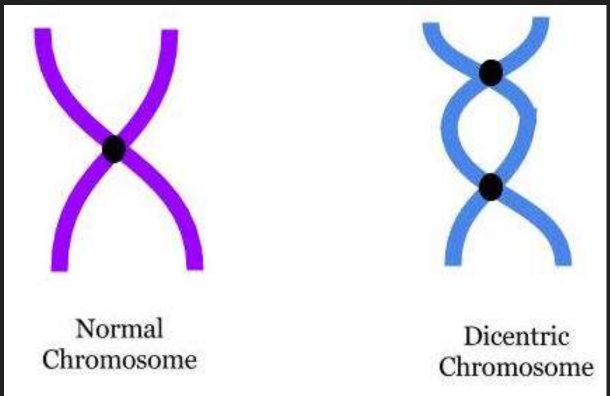
DAPI (nuclear stain)
γH2AX

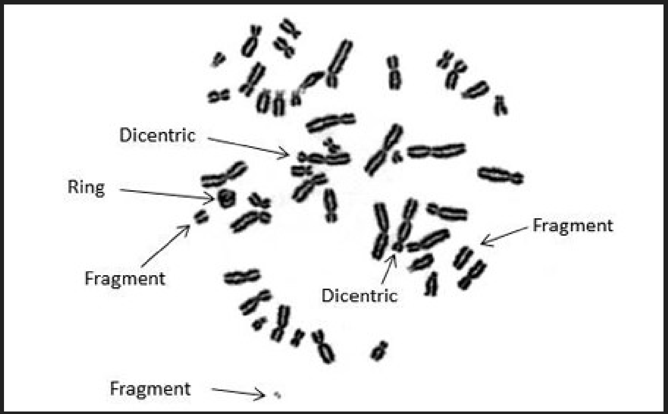
Dicentric assay



- Dicentric (chromosome having two centromeres – constriction in the chromosome that aids in cell division)
- Formed as a result of DNA damage



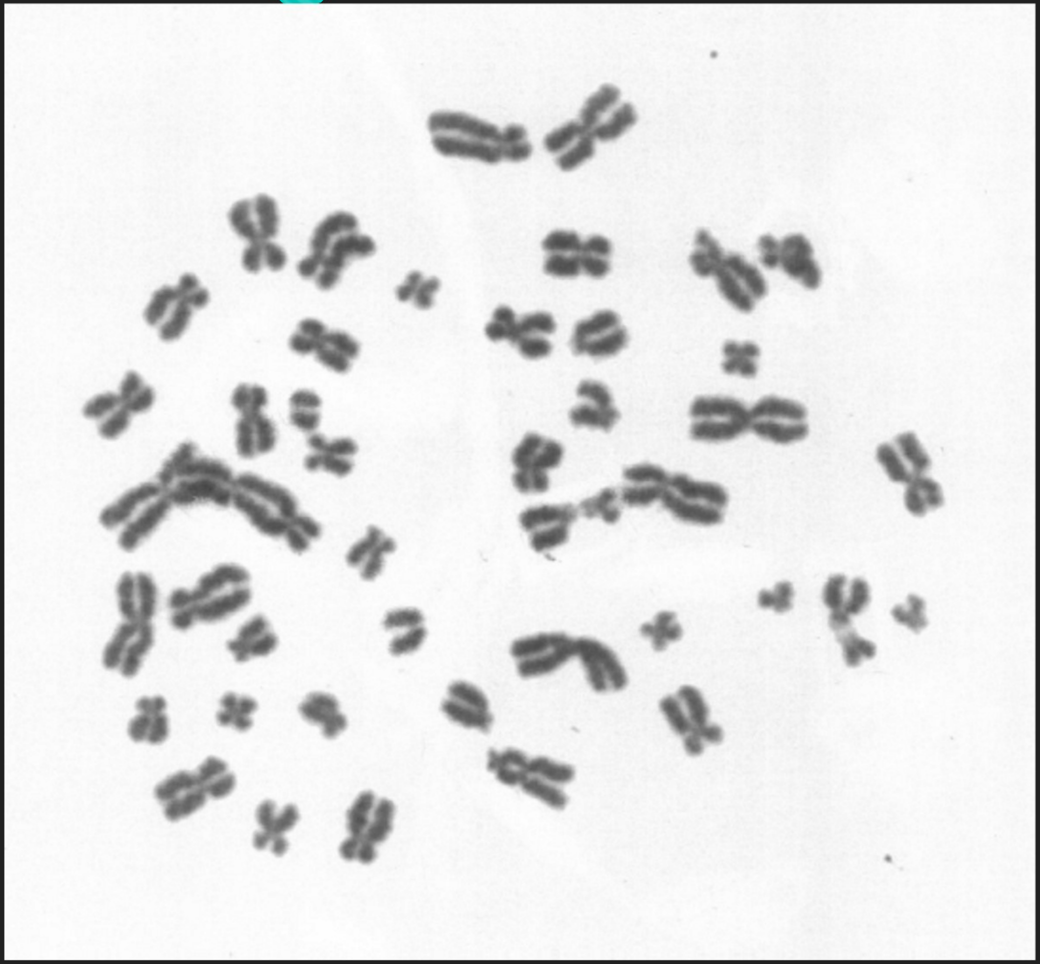


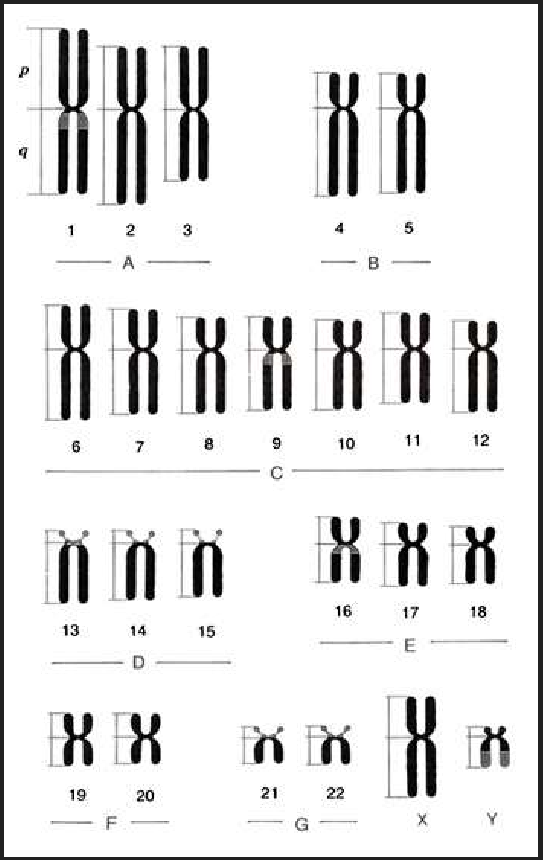


https://study.com/academy/lesson/dicentric-chromosome-definition-example.html

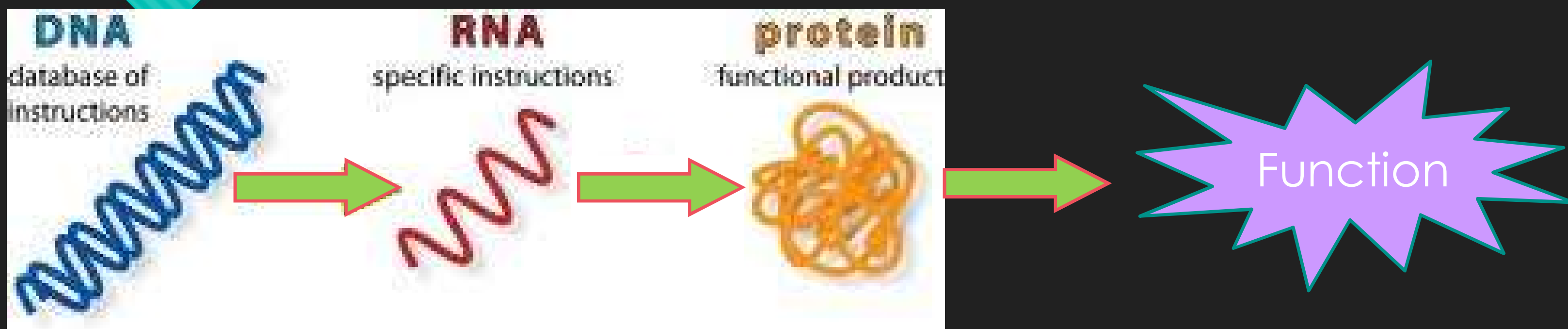
http://www.nirs.qst.go.jp/ENG/core/rmd/05.html

Dicentric assay





Changes in mRNA and protein levels



EVENT

Increased gene expression

Increased amount of mRNA

Increased amount of protein

Upregulated cell function

	No. of copies of mRNA		Differential expression (↑/↓)
mRNA Gene (e.g.)	Control	Exposed	
mRNA1	10	5	↓
mRNA2	2	6	↑
mRNA3	40	40	-
etc..			
mRNAn	40	120	↑

Changes in mRNA expression

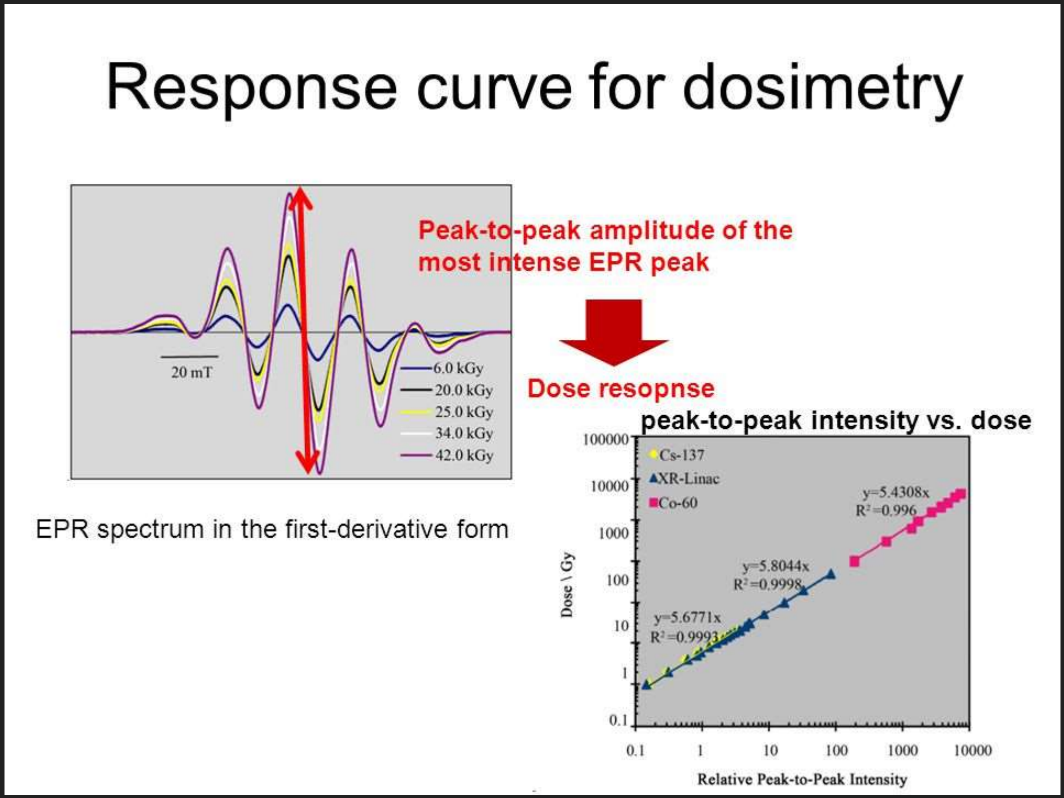
- Blood from 5 male, 3 female, age 26-59 yrs
- Chronic or acute X-ray exposure
- mRNA isolated and analysed
- Different dose rates results in different gene expression profiles (even if same total dose delivered)

Chronic	Acute
mRNA involved in DNA repair - XPC - DDB2 - POLH - GADD45A - PCNA	mRNA involved in metabolism - LDHA - GAPDH - PKFP - ENO1

Ghandhi et al. (2015) *BMC Medical Genomics* 8:22

Electron paramagnetic resonance (EPR)

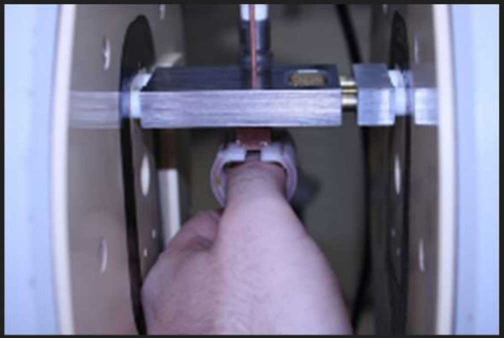
- Magnetic resonance technique used to detect and quantify unpaired electrons
- Free radicals with unpaired electrons generated by exposure to ionizing radiation can be trapped for a long time in calcified tissues
  - lattice crystalline structure of hydroxyapatite in teeth
  - a-keratin in fingernails and hair
- The quantity of unpaired electrons linearly correlates with dose received



http://slideplayer.com/slide/5763365/

Evaluation of EPR as a dosimetric tool

- Advantages
  - Non-invasive
  - Not affected by biological processes such as stress or inflammation hence not affected by simultaneous damage
  - Measurements can be made at any interval after irradiation up to at least 2 weeks (fingernails) or indefinitely (teeth)
- Disadvantages
  - Time consuming
  - Labour intensive
  - Technical expertise required



https://www.dartmouth.edu/~eprctr/research/

Why study biological effects of radiation?

- Influence on policies
- Use in biodosimetry (assessment of radiation dose)
- **Impact on ecology**
- Implications in medicine

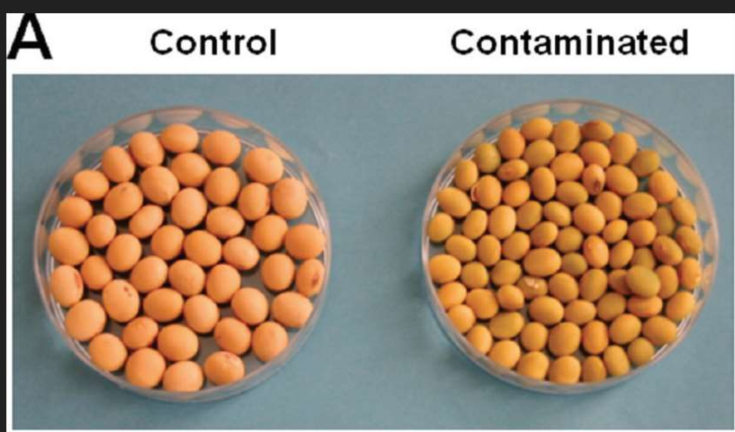
Impact on ecology

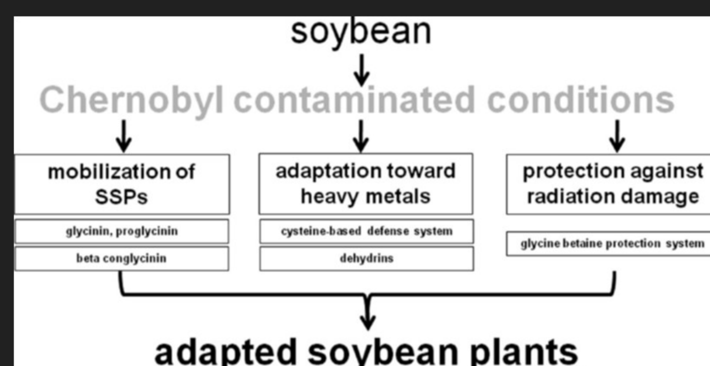
- Chernobyl post-incident studies (10 yrs)
- "No deleterious effects of radiation could be observed in locations where radiological doses were less than or equal to 0.05 Gy/year.
- Where doses between 0.05 and 4 Gy/year were received, radiation effects were 'ecologically masked,' meaning that adverse effects on individual organisms were observed but no changes in populations or ecosystems occurred.
- Where doses were >4 Gy/year, damaging effects on populations and communities occurred."

L. W. Barnthouse, "Effects of Ionizing Radiation on Terrestrial Plants and Animals: a Workshop Report," Oak Ridge National Laboratory, ORNL/TM-13141, December 1995.

Impact on ecology: adaptation

- Soybean seeds from Chernobyl area in contaminated and control areas were harvested and their proteins were analysed
- Several proteins were found to be elevated in plants grown in the contaminated areas
- These proteins were involved in adaptation to heavy metal stress, protection against radiation damage, and mobilization of seed storage proteins





Dachenko et al., (2009) *J Proteome Res* 8:2915-2922

Biological effect of radiation on other organisms

Lethal radiation doses (Gray)		
Organism	Lethal dose	Class/Kingdom
	4–10	
	4.5–12	
	60	
	640	
	15,000	
	30,000	

E. coli

Homo sapiens

Mus musculus

D. radiodurans

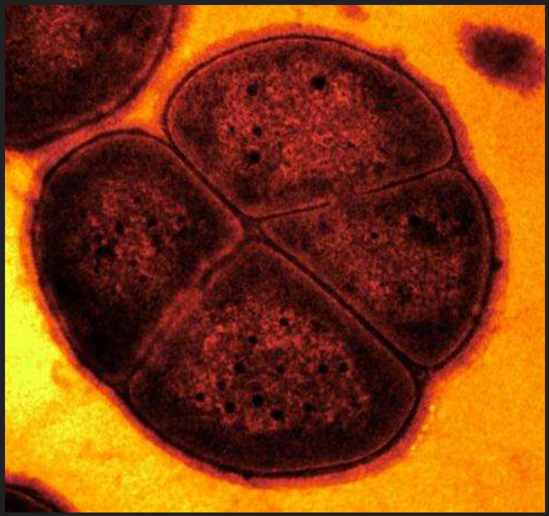
Drosophila sp.

T. gammatolerans

https://en.wikipedia.org/wiki/Radioresistance

Deinococcus radiodurans and its applications

- Mechanisms of radio-resistance:
  - Efficient DNA repair mechanism
  - High levels of manganese that act as anti-oxidant mechanisms
- Potential uses
  - Treatment of nuclear energy waste (genetically modified to digest of heavy metals)
  - Study into mechanisms of DNA repair and anti-oxidant mechanisms for medical benefits



http://bioweb.uwlax.edu/bio203/s2008/arnold_meag/Characteristics.htm

Why study biological effects of radiation?

- Influence on policies
- Use in biodosimetry (assessment of radiation dose)
- Impact on ecology
- **Implications in medicine**

Impact on medicine: radioprotectants

- **Free radical scavengers** e.g. amifostine
- **Boosters of DNA repair** e.g. resveratrol (found in red grapes) stimulates sirtuins (proteins involved in DNA repair)
- **Replenishing lost cells** e.g. Growth factors like granulocyte-colony stimulating factor (G-CSF), hematopoietic stem cell transplantation
- **Protectants of neighbouring cells** e.g. drugs which inhibit inflammatory signals

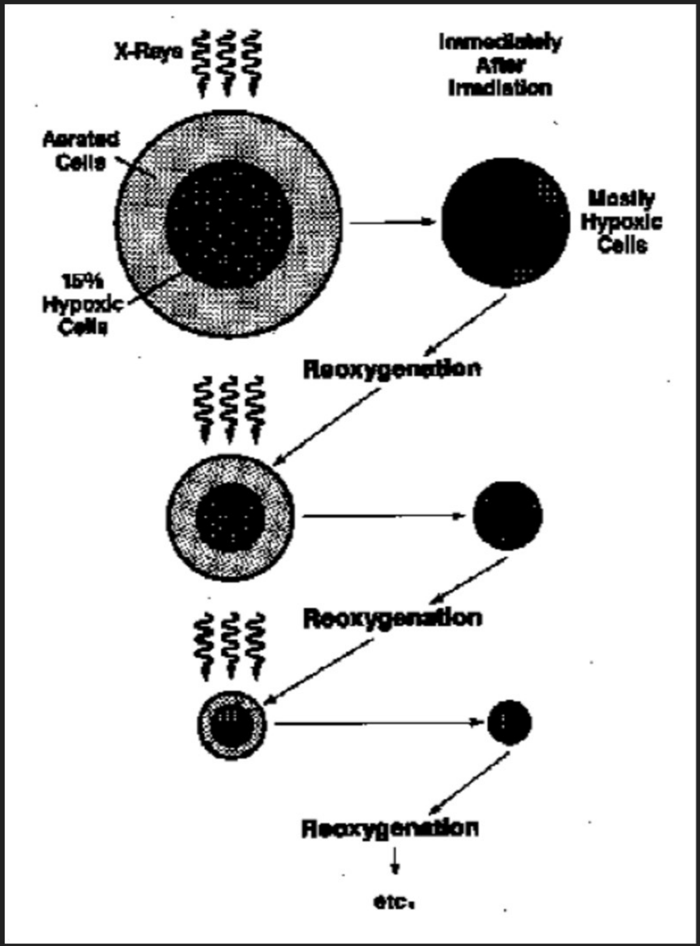
Impact on medicine: radioprotectants

- **Protecting the thyroid**
  - Thyroid gland absorbs iodine from the bloodstream to produce hormones. Radioactive iodine is therefore particularly damaging to the thyroid. Potassium iodide can be administered to saturate binding sites to prevent incorporation of radioactive iodine

- **Blocking death signaling pathways?**
  - Consideration: may prevent acute effects but may be detrimental in the long run as stochastic effects may accumulate

Impact on medicine: radiotherapy

- Sensitizing tumours for more effective therapy
  - Cells deficient in DNA repair enzymes are generally more radiosensitive. Drugs that inhibit DNA repair in tumours are radiosensitizers that can make radiotherapy more effective
  - Hypoxic cells are radioresistant. The core of tumours are generally hypoxic as there is limited supply of oxygen to the centre. Such tumours are therefore difficult to treat with a single targeted dose. Fractionated doses of radiotherapy are believed to be more effective as they gradually erode away at the tumour



https://courses.ecampus.oregonstate.edu/ne581/six/index.htm

Questions?