بسم الله الرّحمان الرّحیم

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عنوان:*اثرات پرتوهای یونیزان بر اندامکهای سلولی*

Effects of ionizing radiations on cellular organelles

گردآوری و ارائه:نیما جعفری رستگار

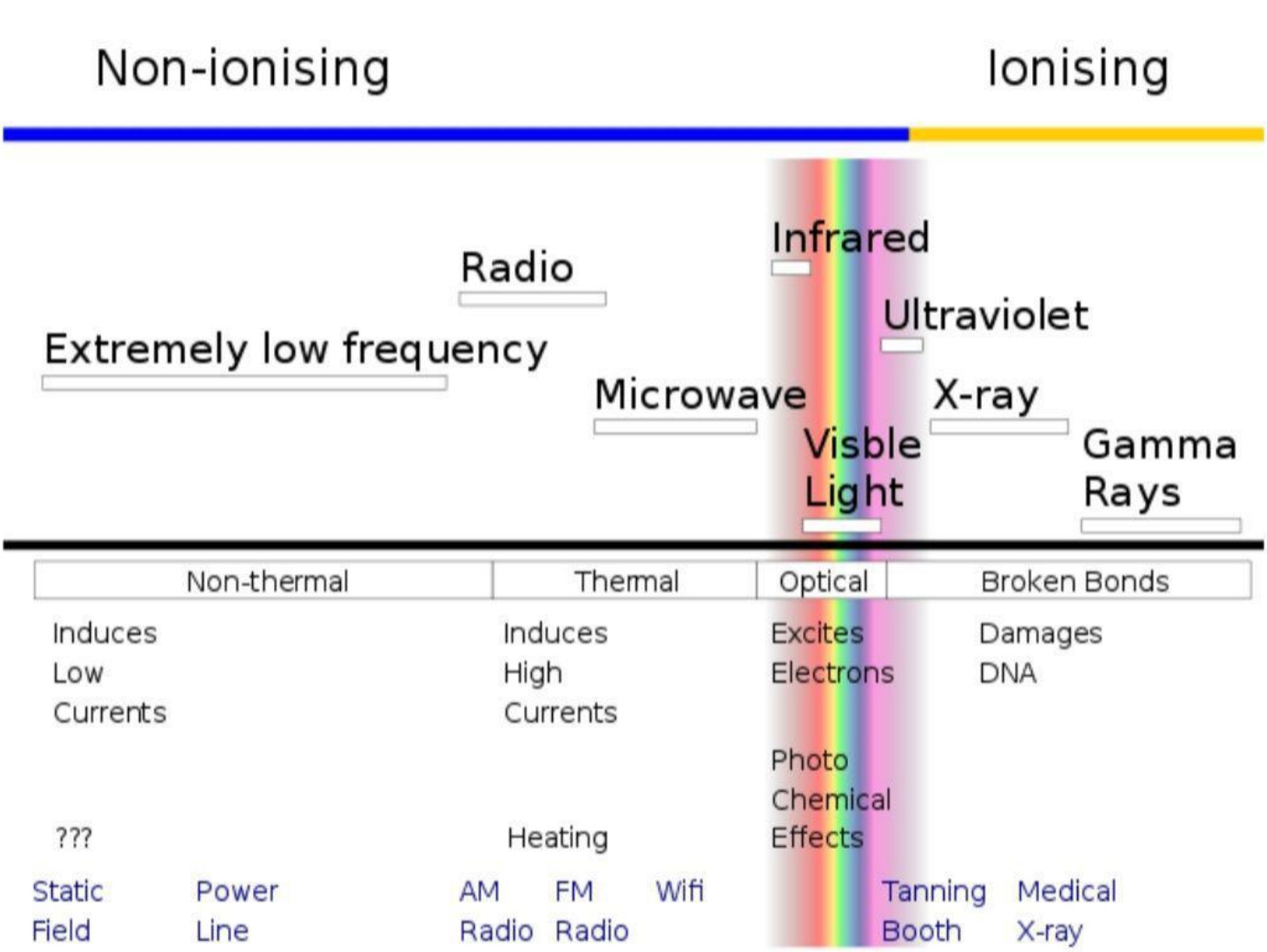
INTRODUCTION

- **Ionizing radiation** is a type of energy released by atoms that travels in the form of electromagnetic waves (gamma or X-rays) or particles (neutrons, beta or alpha).

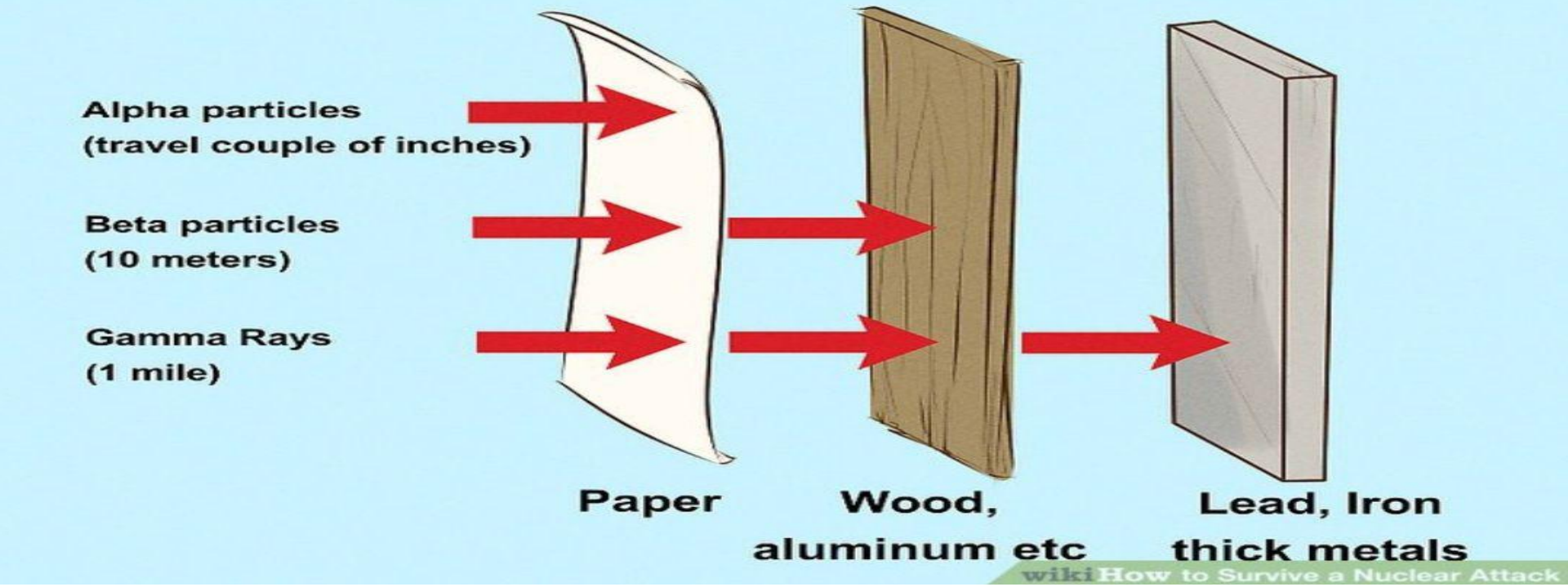
- natural sources of ionizing radiation:in soil, water…
- human-made sources:x-rays and medical devices.

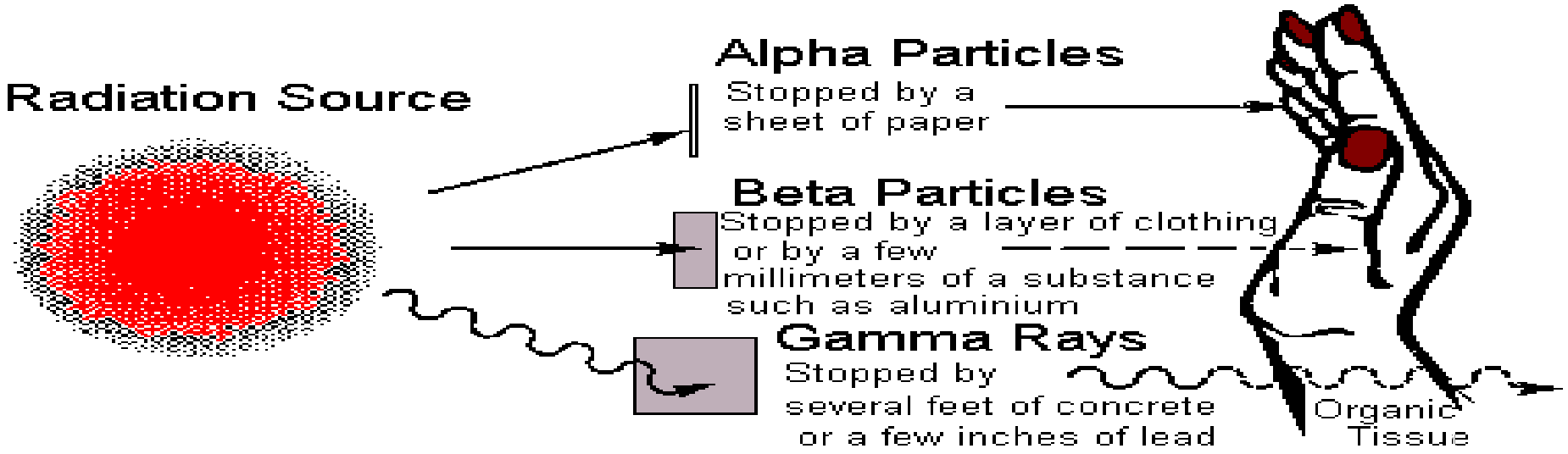
- Acute health effects such as skin burns or acute radiation syndrome can occur when doses of radiation exceed certain levels.

- Whereas **Low doses** of ionizing radiation can increase the **risk of longer term effects** such as cancer.


Non-ionising
Ionising
Infrared
Radio
Ultraviolet
Extremely low frequency
Microwave
X-ray
Visble Light
Gamma Rays
Non-thermal
Thermal
Optical
Broken Bonds
Induces Low Currents
Induces High Currents
Excites Electrons
Damages DNA
Photo Chemical Effects
???
Heating
Static Field
Power Line
AM Radio
FM Radio
Wifi
Tanning Booth
Medical X-ray

Radiation Particles





SOURCES OF IONIZING RADIATION

- There is more than **60 naturally-occurring** radioactive materials found in soil, water and air. **Radon**, a naturally-occurring gas, emanates from rock and soil and is the main source of natural radiation.
- People are also exposed to natural radiation from **cosmic rays**, particularly at high altitude.geographically in certain areas it can be more than 200 times higher than the global average.
- Human-made source of radiation include nuclear power and medical treatments.
  iridium-192 =in industries
  Iodine-131=thyroid cancer
  Iodine-125=thyroid scan
  Technetium-99m=bone scan
  medical research=carbon-14, tritium, and phosphorus-32
  the nuclear fission products such as cesium-137 and activation products such as cobalt- 60)
- Today, the most common one is X-ray machine.

IR IMAGING

- X ray:mammography and fluoroscopy
- CT/CAT scan:same as xray
  nuclear medicine scan: camera + radioactive material
- Based on the patients tolerance and living environment such imaging should only be done when absolutely necessary and never in short periods

Imaging Test	Use Ionizing Radiation	Do Not Use Ionizing Radiation
X-Rays	✓	
MRIs		✓
CT Scans	✓	
Ultrasounds		✓
Nuclear Medicine Scans	✓	

IONIZING RADIATION UNITS

- Radiation dose:the gray (Gy).
- The damage depends on the type of radiation and the sensitivity of different tissues and organs.

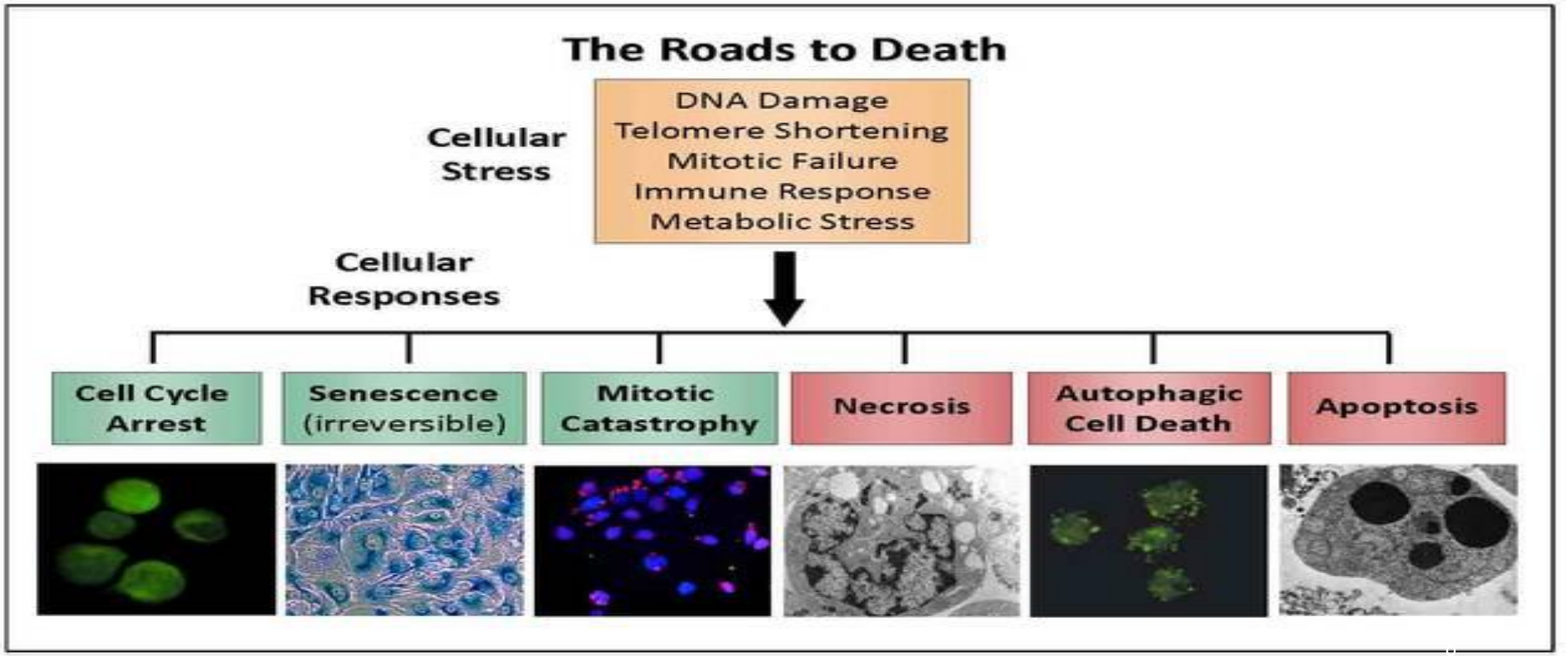
- The effective dose:
  The unit is called The sievert (Sv) which takes into account the type of radiation and sensitivity of tissues and organs.

- Radiosensitivity is the probability of a cell, tissue, or organ suffering an effect per unit dose of radiation.

it is **highest** in cells which are **highly mitotic or undifferentiated**. For this reason the basal epidermis, bone marrow, thymus, gonads, and lens cells are highly radiosensitive. Muscle, bones, and nervous system tissues have a relative low radiosensitivity.

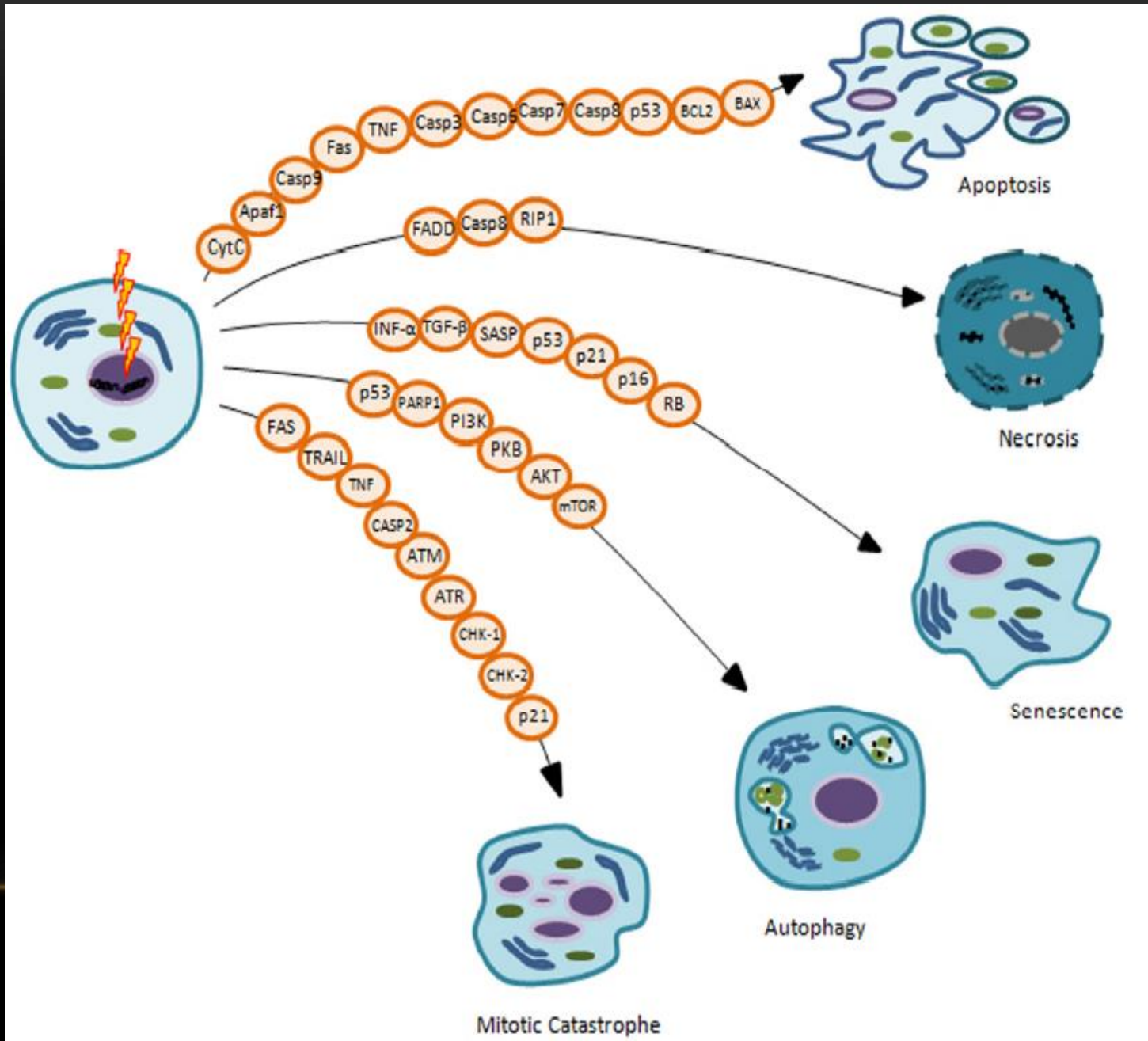
CELLULAR DAMAGE

- The cell might have some minor damage that remains inactive until another agent interacts with the cell again.
- The cell (sperm or egg cell) might have been damaged genetically that doesn't show up until future generations like cancer etc.
- The cell may simply stop functioning and die



MODES OF CELL DEATH AFTER RADIATION

- Apoptotic death
- Necrotic death
- Senescence
- Autophagy
- Mitotic death



MECHANISMS

Mitotic:

-**failure** of **spindle formation**

-**loss of G2 checkpoint**

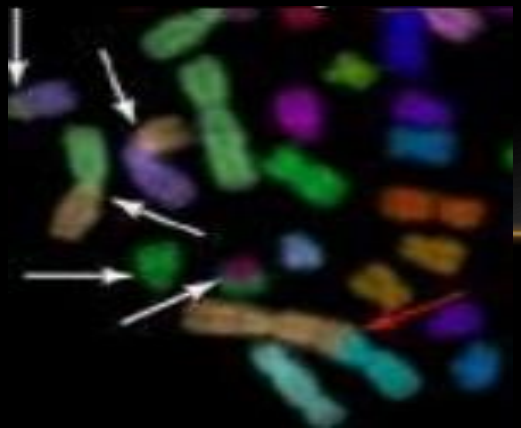
-improper chromosomal segregation

Interphase:

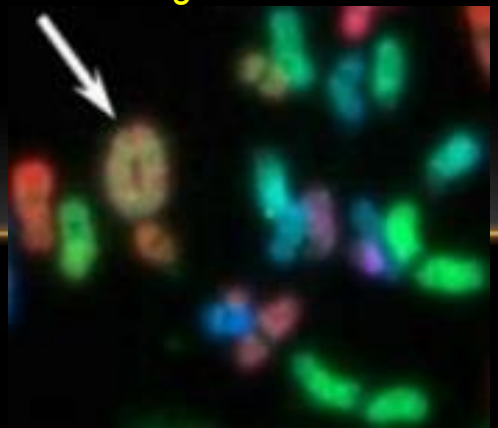
-radiosensitive cells die within 2-6hrs of radiation by **rapid apoptosis**

Lethal chromosomal aberrations :DNA breaks and reassembles incorrectly

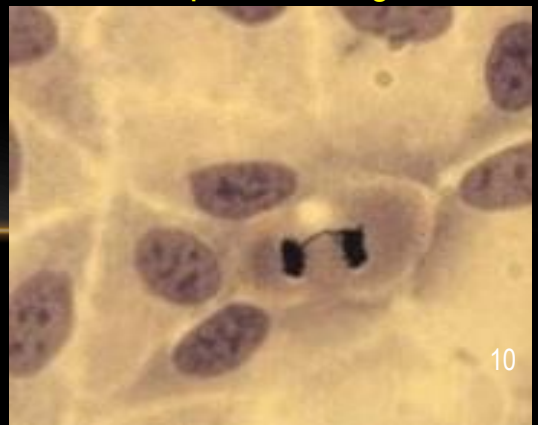
1.Dicentric chromosome

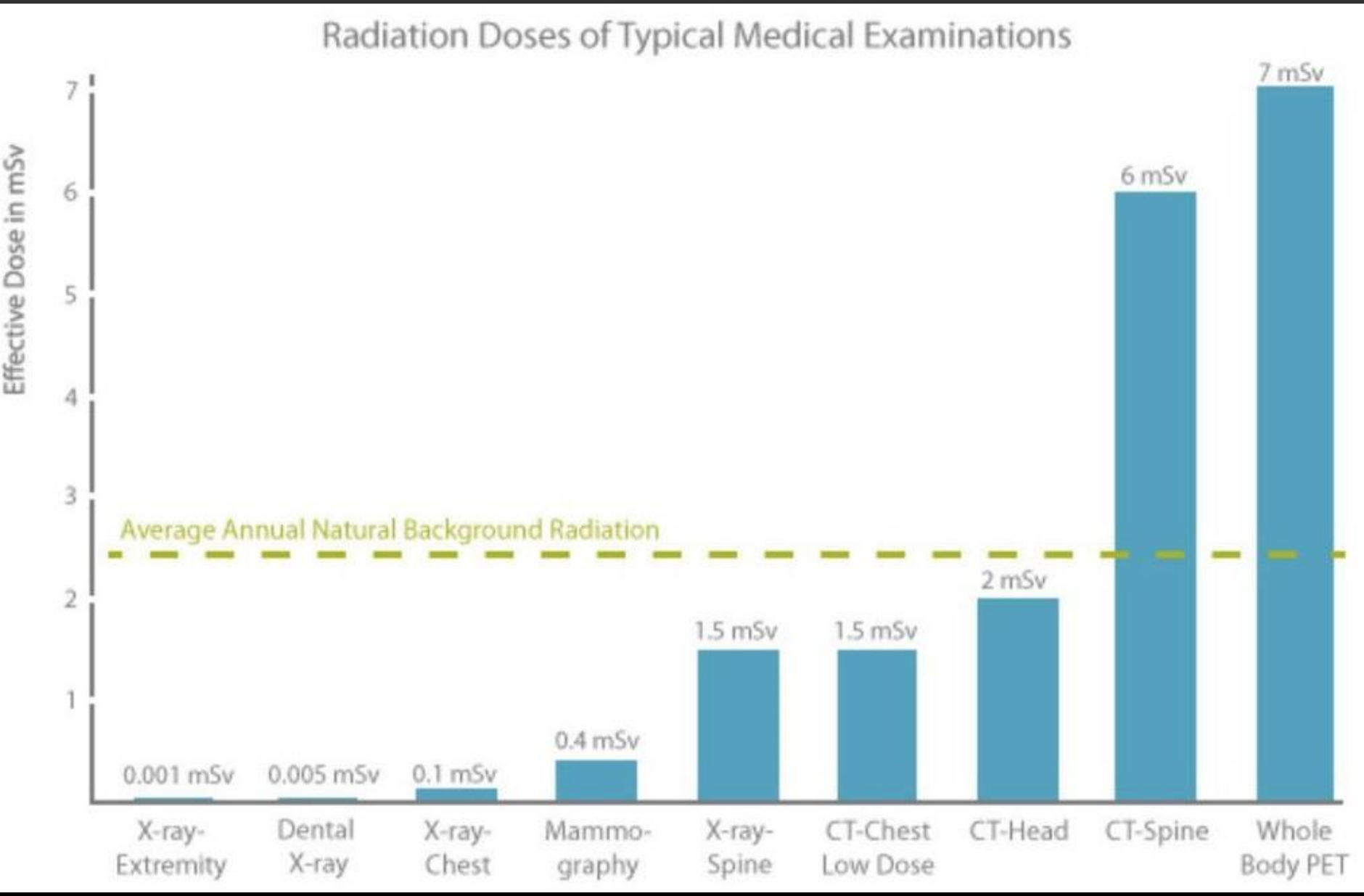


2.Ring chromosome



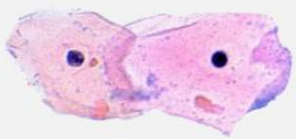
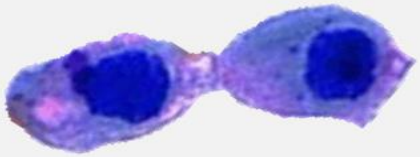
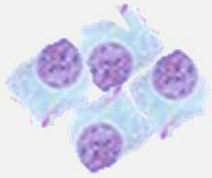
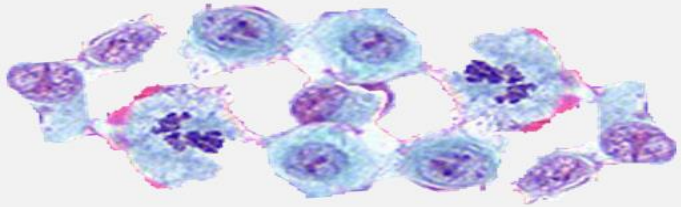
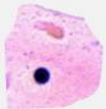
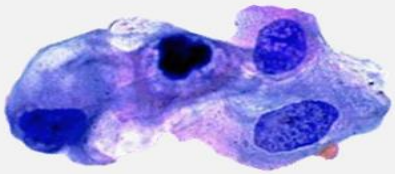
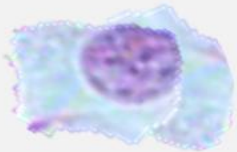
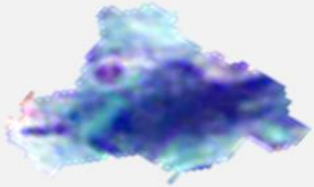
3.Anaphase bridge



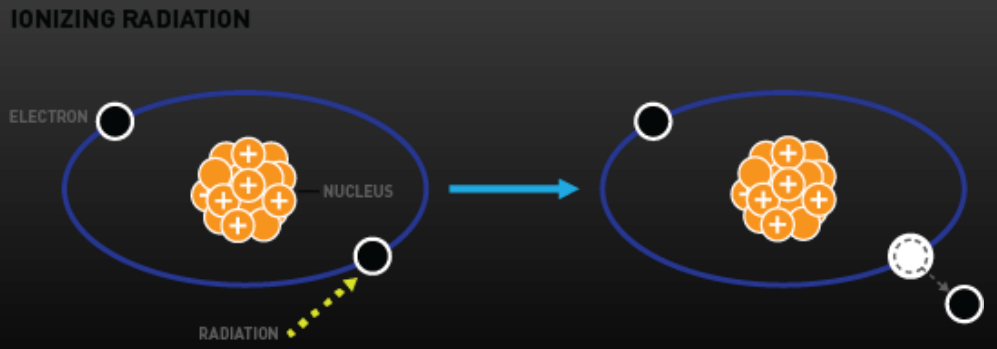

Radiation Doses of Typical Medical Examinations
Effective Dose in mSv
7
6
5
4
3
2
1
Average Annual Natural Background Radiation
0.001 mSv
0.005 mSv
0.1 mSv
0.4 mSv
1.5 mSv
1.5 mSv
2 mSv
6 mSv
7 mSv
X-ray-Extremity
Dental X-ray
X-ray-Chest
Mammo-graphy
X-ray-Spine
CT-Chest Low Dose
CT-Head
CT-Spine
Whole Body PET

NOTE ABOUT CANCER

- Cancers that are actually caused by radiation are completely indistinguishable from those from other causes.scientists observe an exposed group then compare the results to non exposed groups.

Normal	Cancer	
		Large, variably shaped nuclei
		Many dividing cells; Disorganized arrangement
		Variation in size and shape
		Loss of normal features

DAMAGE TO DNA



ionizing radiation contains sufficient energy to displace an electron from its **orbit around a nucleus**.

- **DIRECT DAMAGE** occurs when the displaced electron hits and breaks a DNA strand.
- **INDIRECT DAMAGE**.

A **SSD break** is usually **repaired** appropriately

A **DSD break** has the potential for abnormal reconnection of the strands

(causing all the adverse biological effect)

GAMMA RADIATION OF CELLULAR WATER

rapidly generates

- reactive oxygen species (ROS)
- hydroxyl radical (OH)
- ionized water ($H2O^+$)
- hydrogen radical (H^+)
- hydrated electrons (e^- *aq*)

Secondary products (1 pico second)

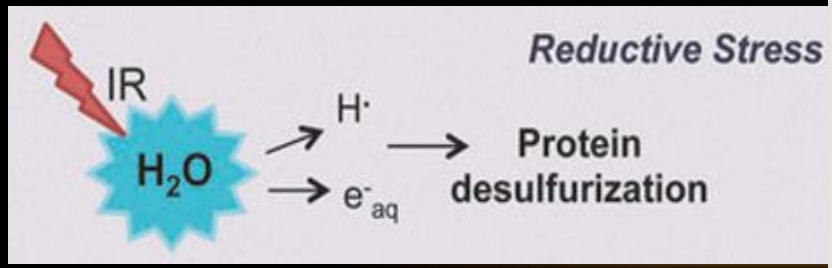
- superoxide (O_2^-)
- hydrogen peroxide (H_2O_2)

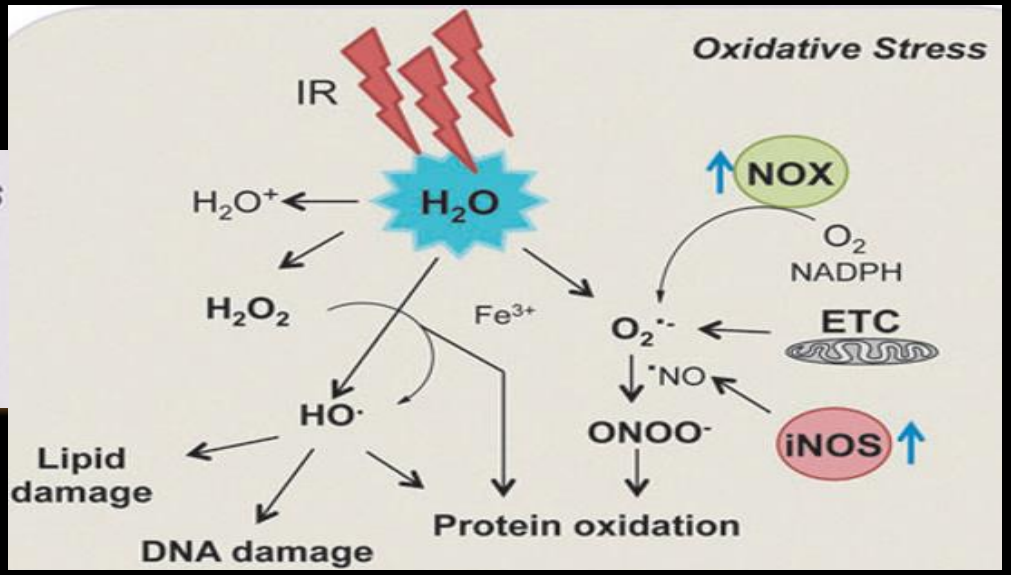
Subsequent chemical Cascades

- metal catalysis:intracellular ferrous and/or cuprous ions convert(O_2^-)and(H_2O_2) into additional ***OH***
- (O_2^-)+ endogenous nitric oxide (NO)=peroxynitrite anion ($ONOO^-$) ⟶ RNS
- **(NO)** also ***directly modifies*** tyrosines,cysteines and hemes

THE TIMING ATTRIBUTES OF CELLULAR DAMAGE INFLICTED BY IR

- chemical reactions = as rapidly as ***0.01 ps after***
- Direct radiation damage is **initiated in the range of 10 s** with the **breaking of S–H,O–H,N–H,and C–H bonds**.
- Damages done by water radicals begin within 1 ps along with thiol depletion and further bond breaking (C–C and C–N).
- By 1ms after IR exposure, the reactions of nascent OH, H, and e^- (aq) are mostly completed ➡ **DNA repair processes are initiated**
- Important event=10 s post-irradiation the increase of intracellular formation of ROS and RNS.
- **Reductive stress** (induced by IR) leads to loss of sulfur in **methionine and cysteine residues**.





IR EXPOSURE LINKED TO MITOCHONDRIA-DEPENDENT ROS/RNS GENERATION IN TUMOR CELLS

- Whole body irradiation of rats:resulted in the increased activity of cytochrome oxidase and NADH-cytochrome c reductase, decreased antioxidant activity, and increased lipid peroxidation in live mitochondrial fractions.

- Irradiation of A549 cells *induced mitochondrial* **ROS production(**can be blocked by respiration inhibitors),**increased membrane potential**,and **promoted respiration and ATP production**.

- An **increased expression of NADPH oxidase** was reported after irradiation with 10 Gy in rat brain microvascular endothelial cells, and. its inhibition led to a decrease in IR-generated ROS it also **reversed** the IR-induced chromosomal instability in hematopoietic stem cells(6.5 Gy)

THE ROLES OF ANTIOXIDANT

- They are paramount even under normal cellular conditions to keep ROS levels in check.

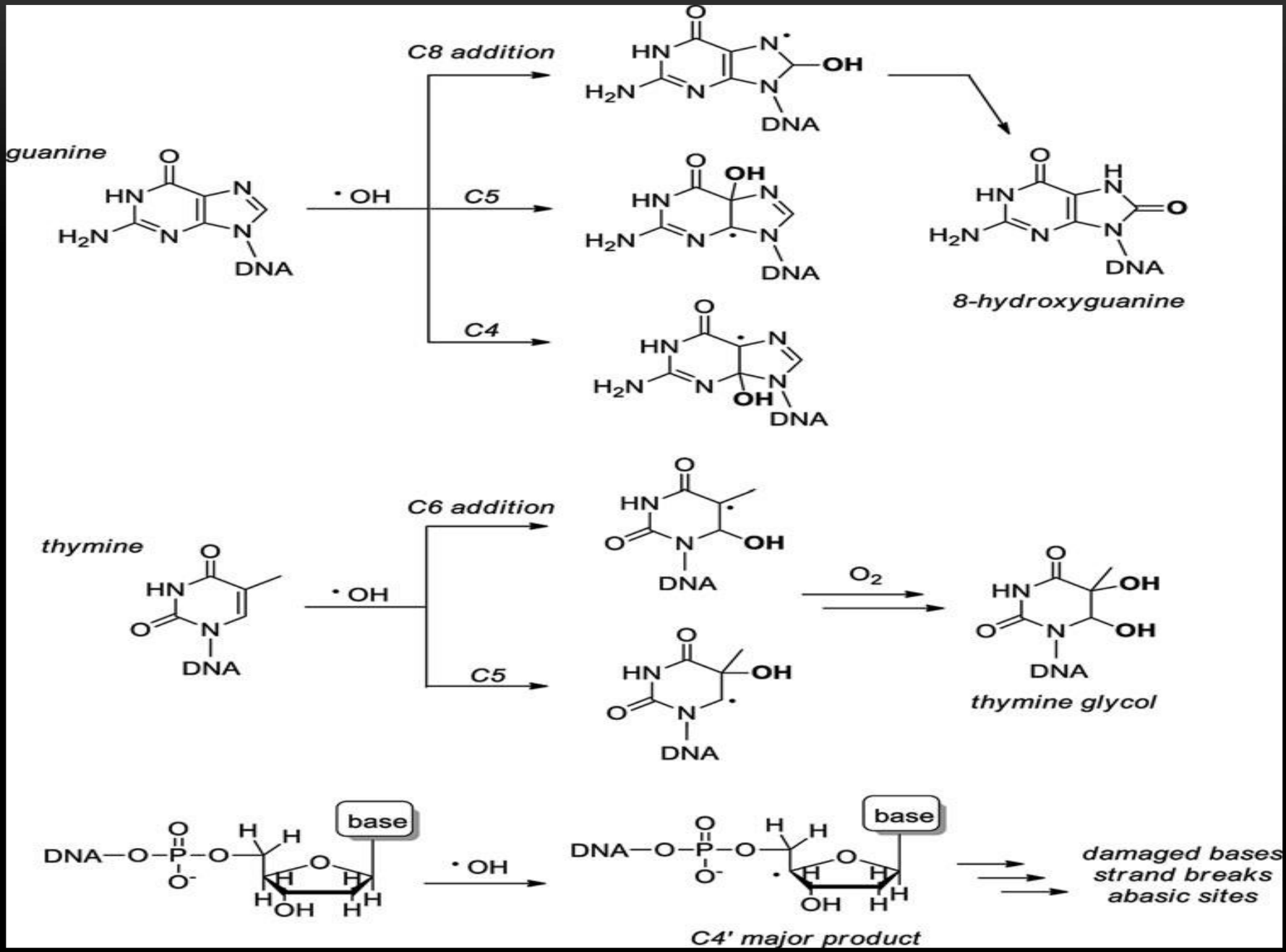
- **Aiding** in this cause are low-molecularweight (MW) endogenous antioxidants such as glutathione(GSH),ascorbate (Vitamin C),melatonin,lipoic acid,ubiquinone(Coenzyme Q10),and Vitamin E,which target water radiolysis products,partially oxidized biomolecules, and peroxynitrite.

- Almost immediately after cellular exposure to IR, the low MW antioxidant supply becomes compromised,for example,causing a rapid decrease in reduced GSH levels.

- In an effort to combat the oxidative burst,cellular transcription factors, including

(NF erythroid-derived 2)-like 2 and (NF-kB) are activated,resulting in the increased expression of ROS detoxifying enzymes,including catalase and SOD along with GPx, glutathione S-transferase (GST), heme oxygenase-1, and several others.

OXIDATIVE DAMAGE TO NUCLEOBASES

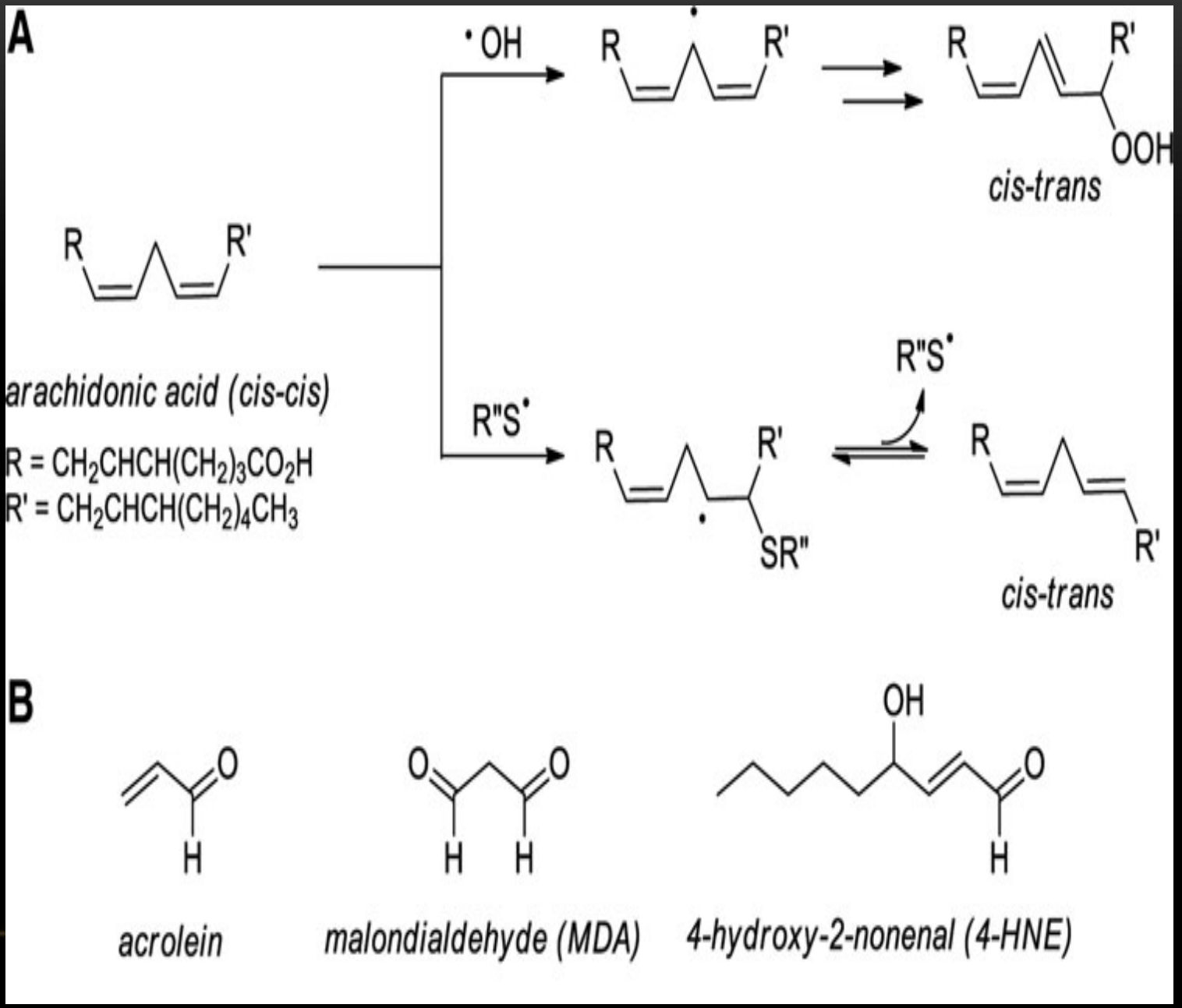


DAMAGES TO THE LIPIDS

- Though radiation is capable of directly damaging lipids,but indirect damage induced by water radiolysis products is more widespread.
- Radiation induces lipid peroxidation, particularly the peroxidation of polyunsaturated fatty acids (PUFAs), leading to an increase in membrane permeability,disruption of ion gradients and altered activity of membrane-associated proteins.
- induction of apoptosis at 5–10Gy occure via increased ceramide Levels.This outcome was observed even in cells without a nucleus.
- **Sphingolipid is altered in response to IR**. The sphingolipid ceramide, a product of sphingomyelin hydrolysis catalyzed by acid sphingomyelinase(ASMase).Cellular exposure to IR leads to ASMase relocalization **from the lysosomes to the plasma membrane**,generating large amounts of ceramide.(DNA damage events activate ceramide synthase)
- ceramide-containing lipid rafts form large membrane platforms which not only contain membrane receptors and proteins but are also enriched in nuclear enzymes such as DNA-PK that are relocalized on irradiation,distrupting many interactions…

(A)
OXIDATIVE
CIS-TRANS ISOMERIZATION OF
POLYUNSATURATED
FATTY ACIDS.

(B)
REACTIVE ALDEHYDES
GENERATED
BY LIPID
PEROXIDATION.



PROTEIN ALTERATIONS

- 2 hrs after Exposing human peripheral lymphocytes ex vivo by irradiating whole blood with gamma doses of 1, 2 and 4 Gy Proteomics analysis was performed (2D-PAGE)This study was able to find only around **20 altered proteins** of which **11 could be identified.**
- Out of **five proteins** showing significant **down-regulation** **four were structural proteins** (beta-actin, talin-1, talin-2, zyxin-2). Also **peroxin-1** involved in protein transport and degradation.
- **Four** of the **up-regulated** proteins were ***associated with cell cycle control*** and immune system.
- **Six** proteins showed an **altered phosphorylation** status (MHC binding protein-2, phosphoglycerate kinase-1, annexin-A6, zyxin-2, interleukin-17E and beta-actin)

MODIFICATION OF AMINO-ACID SIDE CHAINS

- **OH** initiates cleavage of the protein backbone and **reacts** with each of the *twenty standard amino acids and selenocysteine*
- Radicals are believed to preferentially **react with the protein amide** backbone over the amino-acid side chains, readily abstracting hydrogen atoms and **forming a-carbon-centered radicals**.
- **OH** abstracts **hydrogen** atoms from **aliphatic amino-acid side chains at all carbons**(high and chemoselective reactivity)
- **aliphatic amino-acid radicals** are rapidly oxygenated and **generate peroxyl radicals or are repaired by cysteine thiols**, leading to thiyl radicals.
- **Aromatic amino acids** are significantly ***more reactive*** to **OH addition to the aromatic ring**. In the case of ***Tyr***, OH addition and subsequent hydrogen abstraction ***leads*** to ***phenoxyl radicals***,which in the absence of reductants, ***form Tyr dimers*** that are implicated in the formation of ***intra- and inter-protein linkages***.***RNS*** also **targets Tyrosin and Tryptophan.**
- **Radiation-generated ROS** are noted to modify cellular proteins by **carbonylation of amino-acid side chains**,particularly occurring via metal catalysis on Lys, Thr, Pro,Glu, Asp, and Arg residues.note:Repair processes have not been identified,only degradation.
- In total, **methionine oxidation** selectively modulates numerous cell signaling pathways that are regulated by calcium,phosphorylation, and others.

RADIATION-INDUCED SULFUR OXIDATION IN PROTEINS, AFFECTING

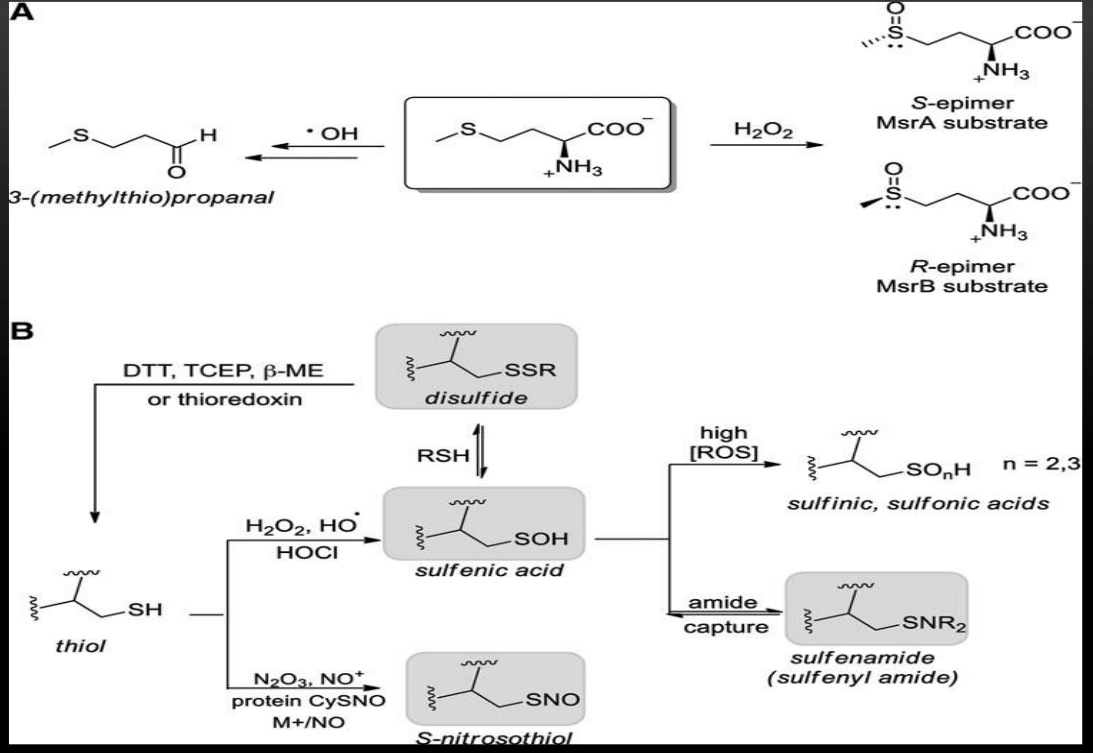
(A) METHIONINE

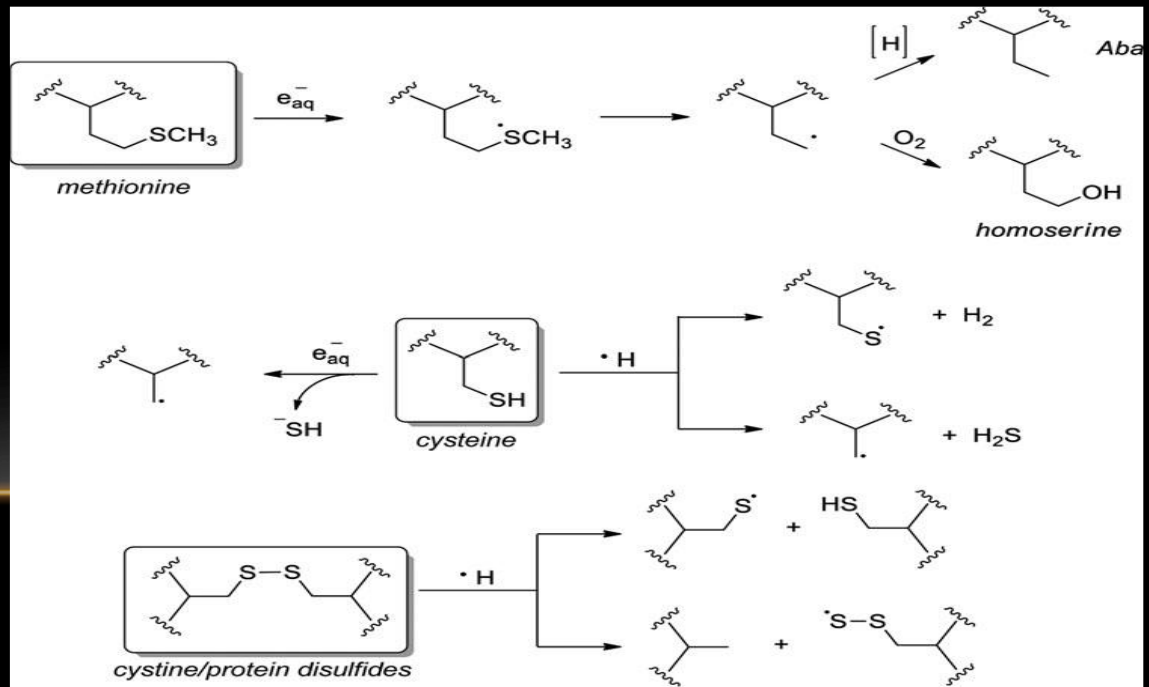
(B) CYSTEINE RESIDUES

AMONG THE AMINO ACIDS, PROTEIN ***CYSTEINE RESIDUES HAVE THE LOWEST REDOX POTENTIAL***

IMPACT OF IR-INDUCED REDUCTIVE STRESS ON PROTEIN SULFUR, INCLUDING METHIONINE, CYSTEINE, AND CYSTINE.

DISULFIDIZATION





AFFECTED PROTEINS

- proteins implicated as sensors and transducers of DNA damage:

the Mre11–Rad50–Nbs1 complex, BRCA1/2, ATM/ATR, DNAPK,checkpoint kinases 1/2 and poly(ADP-ribose) polymerase.(tightly regulated by multiple post-translational modifications)

- **ATM** a key regulator of all checkpoints,is activated by IR in a mechanism

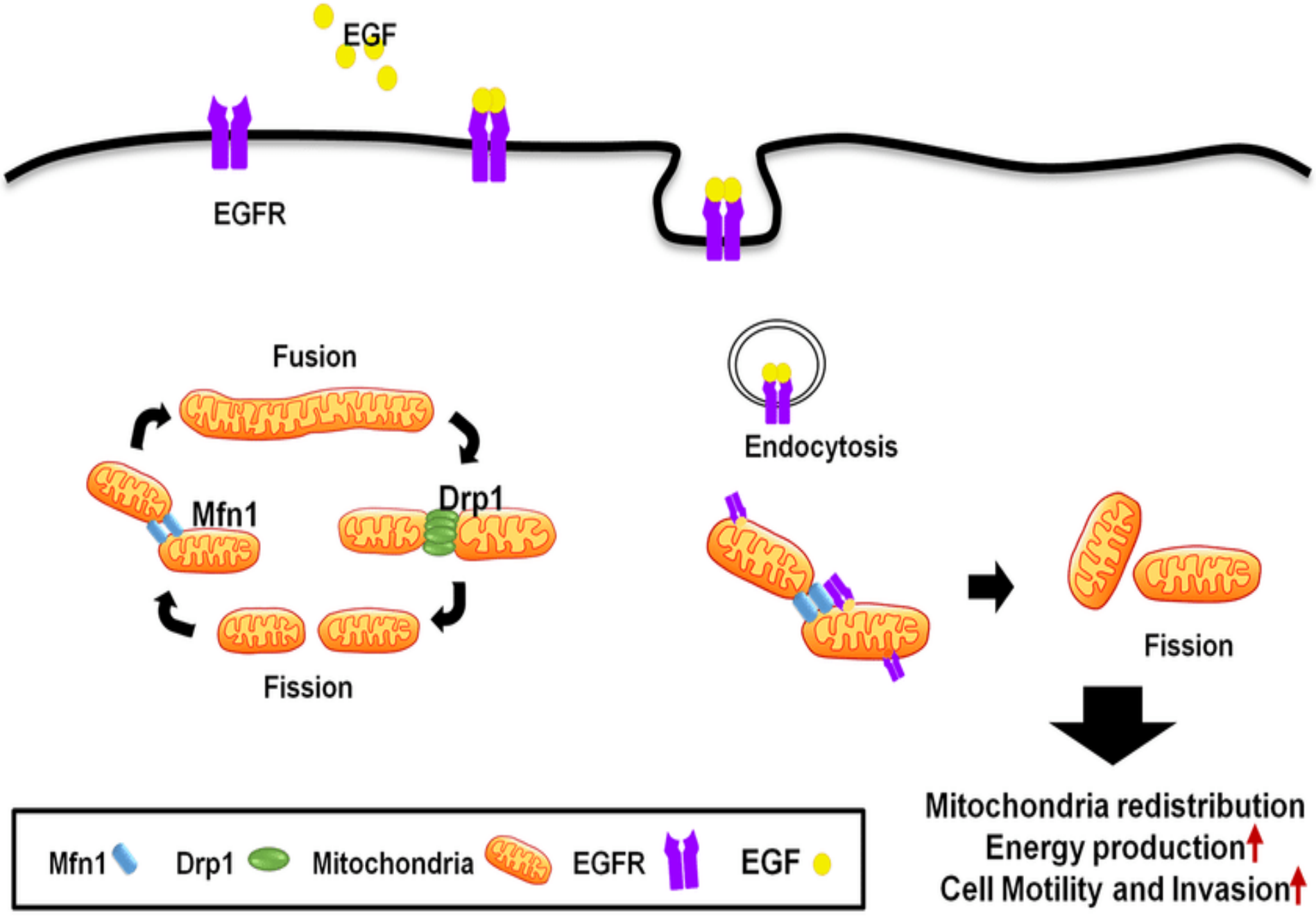
involving phosphorylation at Ser1981(also directly activated by IR-induced ROS)

It facilitates dimerization of intermolecular disulfide and also mediates the phosphorylation of Kap1, promoting heterochromatin relaxation and increasing the efficiency of DNA repair.

- **ATM** was found to regulate **purine, pyrimidine, and urea cycle metabolism** through the ***activation of AMP-activated protein kinase***(a crucial sensing enzyme in the regulation of cellular energy pathways)

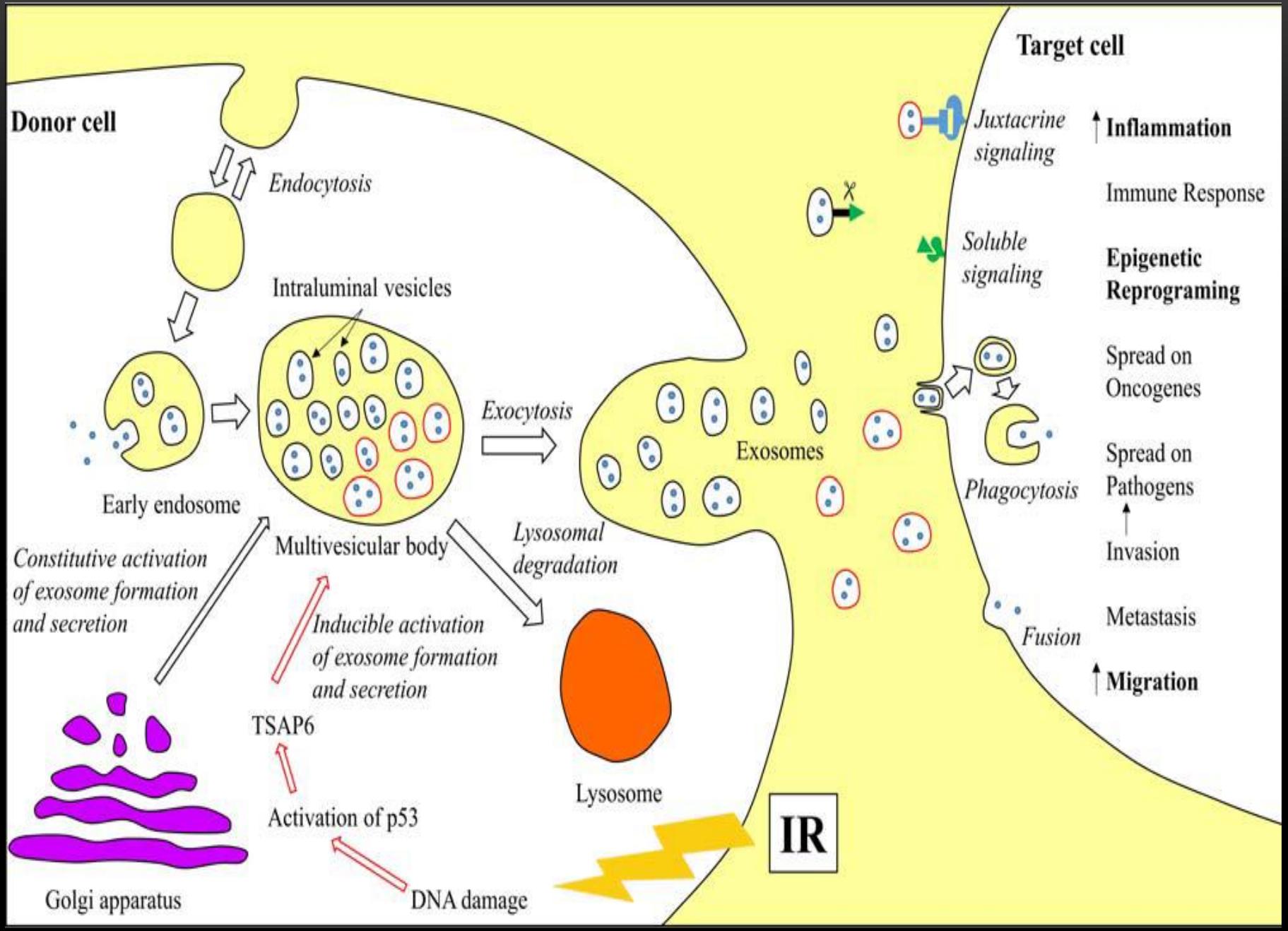
DYNAMICS OF MITOCHONDRIA

- **Fusion**:relies on the inner membrane protein optic atrophy 1 and the outer membrane proteins mitofusin 1 and 2.
- Fission:requires the ***translocation*** of dynamin-related protein 1 (***Drp1***) from the cytosol to mitochondria(on the outer membrane)
- Similar to the motor dynamin, Drp1 is believed to polymerize into ring- or spiral-shaped superstructures that constrict and eventually cleave mitochondria via a GTP hydrolysis–dependent mechanism.
- Drp1 inhibition leads to increased length of mitochondria,thereby inhibiting the fission process and ***preventing mitotic catastrophe***(normal cell contains one or two centrosomes, a cell that underwent MC presents an atypical number of centrosomes)
- IR **stimulates** Drp1 translocation and therefore mitochondrial fission.radiation-induced MC is independent of apoptosis signaling but is ***dependent on PLK 1 and CDK2***(***Essential for centrosome maturation and duplication***)
- Note:when the cell cycle arrest is prolonged due to excessive DNA damage, ***it restarts prematurely***, carrying unrepaired damages,which was suggested to be involved in the events associated with defective or deregulated mitosis.
- Drp1 affects the expression of mitotic regulators after IR,ex:**its deficiency elevates cyclin B1** level after irradiation.


EGF
EGFR
Fusion
Mfn1
Drp1
Fission
Endocytosis
Fission
Mitochondria redistribution
Energy production
Cell Motility and Invasion
Mfn1
Drp1
Mitochondria
EGFR
EGF

EXOSOME SECRETION

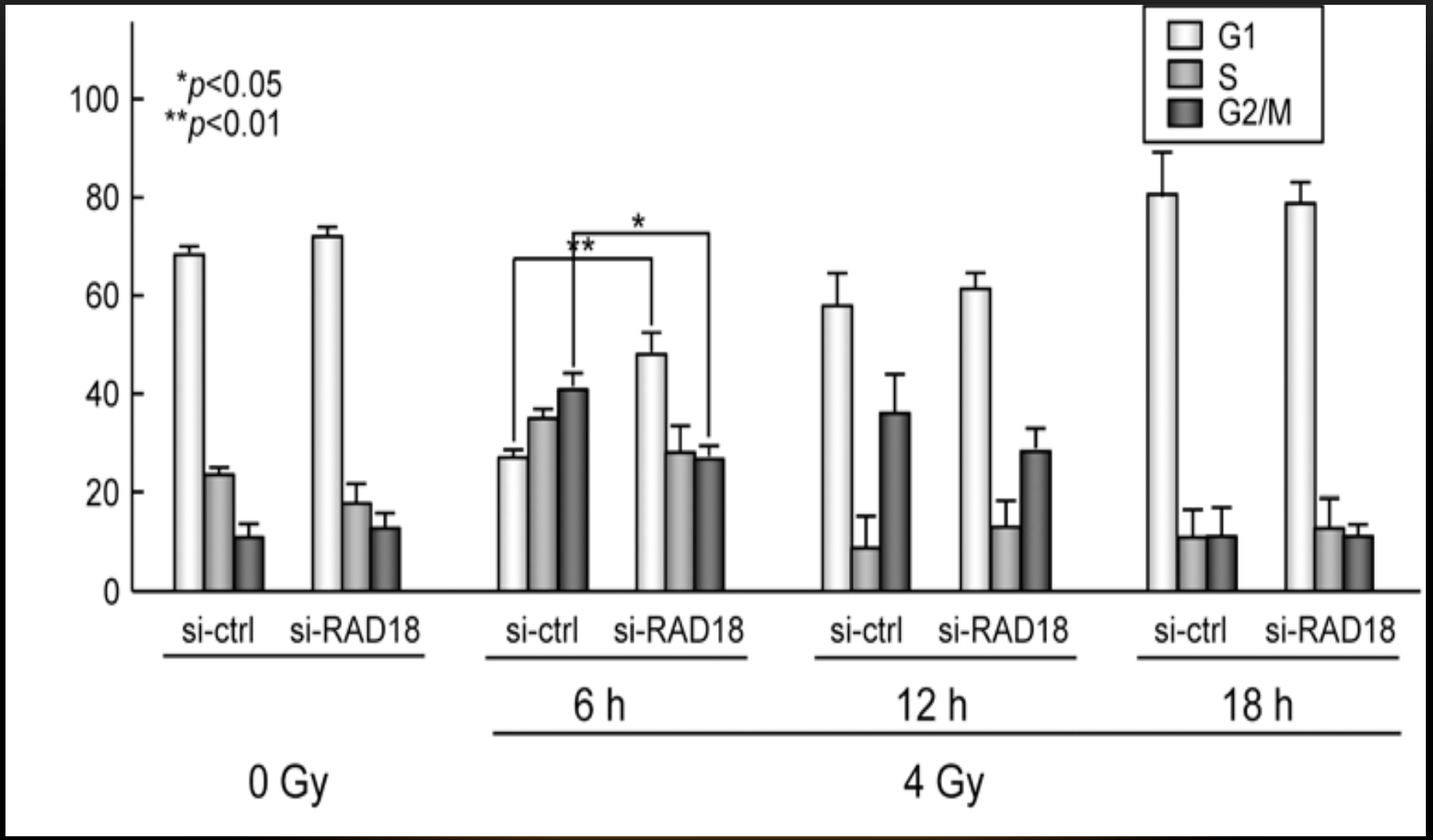
- Inducible pathways of exosome secretion activated in irradiated cells are regulated by TSAP6 protein(the transmembrane protein tumor suppressor-activated pathway 6),which is transcriptionally regulated by ***p53***, hence affecting composition and secretion rate of exosomes from target cells.
- IR influences cell to cell communication through different signal that may be passed between unaffected and/or affected cells and vice versa.
- Exosomal release of unnecessary proteins and RNA/miRNAs instead of their lysosomal processing is beneficial to cells that do not have efficient degradation capability or are located toward a drainage system.
- Some exosomes induce antigen-specific tolerance (tolerosomes).In normal cells they were shown to participate in increasing the tolerance to food antigens in intestines and in enhancing an immune tolerance during pregnancy
- Some exosomes released from tumor cells ***spread oncogenes***.In particular, the oncogenic mutant(EGFRvIII) expressed by glioma cells associated with cancer aggressiveness and transferred in vesicles to other tumor cells missing this mutant receptor.
- They also launch ***downstream anti-apoptotic and angiogenic mediators*** such as (VEGF),leading to independent growth capacity.


Donor cell
Endocytosis
Intraluminal vesicles
Early endosome
Multivesicular body
Exocytosis
Exosomes
Lysosomal degradation
Lysosome
Constitutive activation of exosome formation and secretion
Inducible activation of exosome formation and secretion
TSAP6
Activation of p53
DNA damage
Golgi apparatus
IR
Target cell
Juxtacrine signaling
Soluble signaling
Phagocytosis
Fusion
Inflammation
Immune Response
Epigenetic Reprograming
Spread on Oncogenes
Spread on Pathogens
Invasion
Metastasis
Migration

RAD18

- **RAD18** ***Activates*** ***the G2/M Checkpoint*** through DNA Damage Signaling
- ***RAD18***(ubiquitin ligase) is involved in post replication repair pathways via its **recruitment to stalled replication forks**, and its role in the ubiquitylation of proliferating cell nuclear antigen(PCNA).it is also recruited to (DSB) sites in the DNA damage response induced by IR.
- ***Depletion of RAD18 increased micronuclei formation and cell death following IR exposure.***
- ***RAD18*** **localizes** to DSBs during all phases of the cell cycle and is not affected in cells **deficient** for BRCA1, NBS1 or RAP80, suggesting that RAD18 functions **up stream** of them.
- Cells deficient for Rad18 exhibit increased sensitivity to various DNA damaging agents (and display enhanced genome instability)
- ***Mammalian cells*** use two major DNA repair systems, ***non-homologous end joining (NHEJ) and homologous recombination*** (HR) depending on phases of the cell cycle.
- During the ***late S phase and the G2 phase***, ***HR***, which ***requires a sister chromatid or homologous chromosome*** to use as the repair template, is the predominant repair pathway.
- In the ***G1 phase***, ***RAD18*** interacts with ***53BP1*** to promote DSB repair via the NHEJ pathway.
- the ***recruitment of RAD18*** depends on H2AX, MDC1 and RNF8 in the DNA damage signaling pathway after IR exposure but said recruitment occurs independently of ***RAD18 deficiency***, suggesting that RAD18 functions down stream of them.
- H2AX MDC1 RNF8 RAD18 BRCA1 NBS1 RAP80

G2/M ARREST


*p<0.05
**p<0.01
G1
S
G2/M
100
80
60
40
20
0
si-ctrl
si-RAD18
si-ctrl
si-RAD18
si-ctrl
si-RAD18
si-ctrl
si-RAD18
6 h
12 h
18 h
0 Gy
4 Gy

RESOURCES:

- http://www.who.int/news-room/fact-sheets/detail/ionizing-radiation-health-effects-and-protective-measures

  http://www.who.int/ionizing_radiation/pub_meet/tech_briefings/potassium_iodide/en/

  https://www.arpansa.gov.au/understanding-radiation/what-is-radiation/ionising-radiation/health-effects

  https://www.imagewisely.org/Imaging-Modalities/Computed-Tomography/Imaging-Physicians/Articles/Ionizing-Radiation-Effects-and-Their-Risk-to-Humans

  https://en.m.wikipedia.org/wiki/Ionizing_radiation

  https://www.medicalradiation.com/facts-about-radiation/benefits-and-risks-of-radiation/benefits-and-risks-of-ionizing-radiation/

  http://www.radiationanswers.org/radiation-introduction/radiation-exposure.html

  https://www.researchgate.net/publication/235398217_Ionizing_Radiation_Effects_on_Cells_Organelles_and_Tissues_on_Proteome_Level

ARTICLES

- Effects of Ionizing Radiation on Biological Molecules—Mechanisms of Damage and Emerging Methods of Detection ANTIOXIDANTS & REDOX SIGNALING Volume 21, Number 2, 2014ª Mary Ann Liebert, Inc.(DOI: 10.1089/ars.2013.5489)
- RAD18 Activates the G2/M Checkpoint through DNA Damage Signaling to Maintain Genome Integrity after Ionizing Radiation Exposure OPEN ACCESS (PLOS ONE | DOI:10.1371/journal.pone.0117845 February 12, 2015)
- **Inhibition of the mitochondrial fission protein dynamin-related protein 1 (Drp1) impairs mitochondrial fission and mitotic catastrophe after x-irradiation** Address correspondence to: Osamu Inanami (inanami@vetmed.hokudai.ac)This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E15-03-0181) on October 14, 2015. MBoC | **ARTICLE**
- **The Influence of Ionizing Radiation on Exosome Composition,Secretion and Intercellular Communication** *Address correspondence to this author at the Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Wybrzeze Armii Krajowej 15, 44-100 Gliwice, Poland. Tel: +48 32 278 9627; E-mail:m_pietrowska@io.gliwice.pl