Bystander Effects of Radiation

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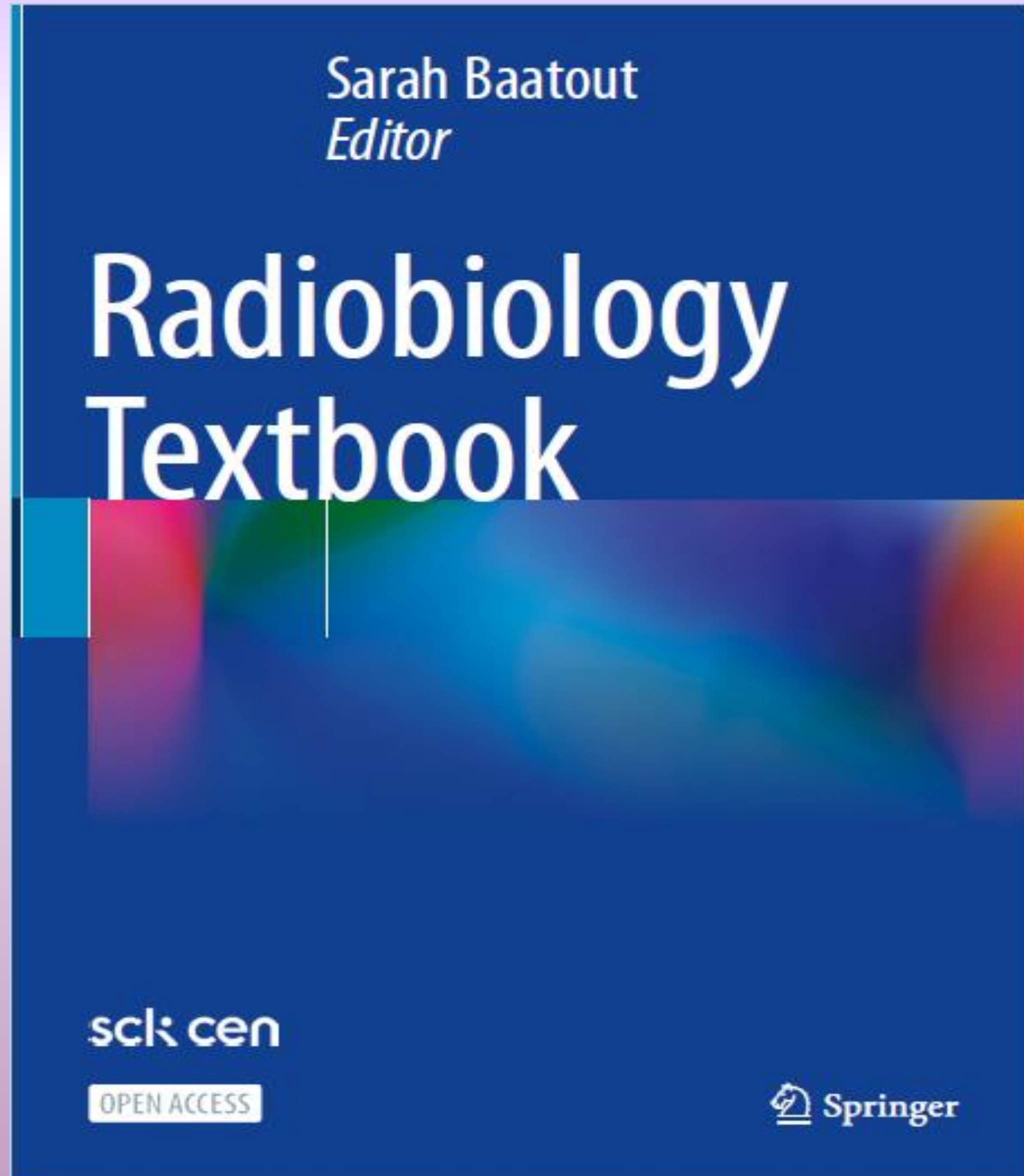
Bystander Effects: OVERVIEW

- Source of Radiation Exposure
- Radiation Effects
- Paradigm shift – Non Targeted Effects
  - Genomic Instability (GI) & Bystander Effects (BE).
  - **BE –definition**
  - BE – evidence (*in vitro & in vivo*)
- Bystander Effects – methods of investigations
- Bystander Effects – possible mechanisms
  - the role of Microvesicales / Exosomes in BE
- Mechanistic Link between GI&BE
- Summary and comments

The radiobiology textbook was published in Oct. 2023 and is freely available to the public at the following link.
https://link.springer.com/book/10.1007/978-3-031-18810-7



WHY IS IONIZING RADIATION RELEVANT?

Natural radiation exposure (85%)

- Cosmic Rays
- Radon Gas
- Food

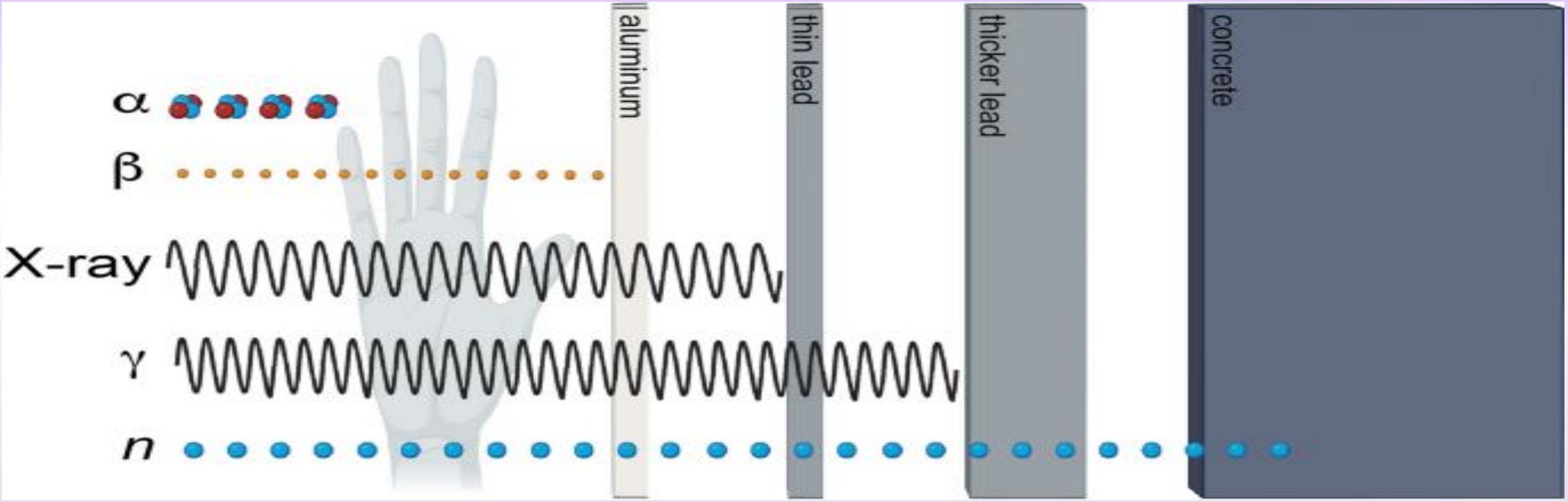
Artificial radiation exposure (15%)

- From Medical Settings for : Therapeutic and diagnostic exposures

Following radiation exposure, cellular events/ biological effects include:

Genetic changes in somatic cells that cause cancer; genetic mutation that affects future generation & radiation death

Characteristics of Variable Penetration radiation are radiation type dependent: (High LET (α-particles) *Vs* Low LET (x, β & Gamma γ)



aluminium lead concrete

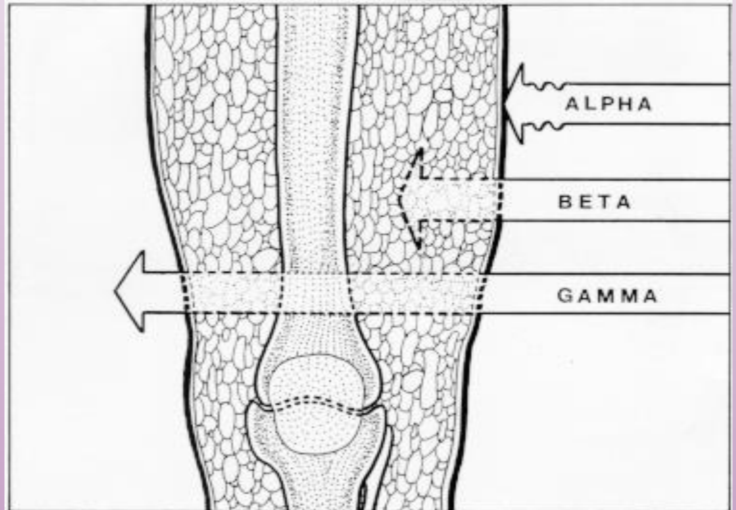


Figure 3. Radiation travelling through human tissue

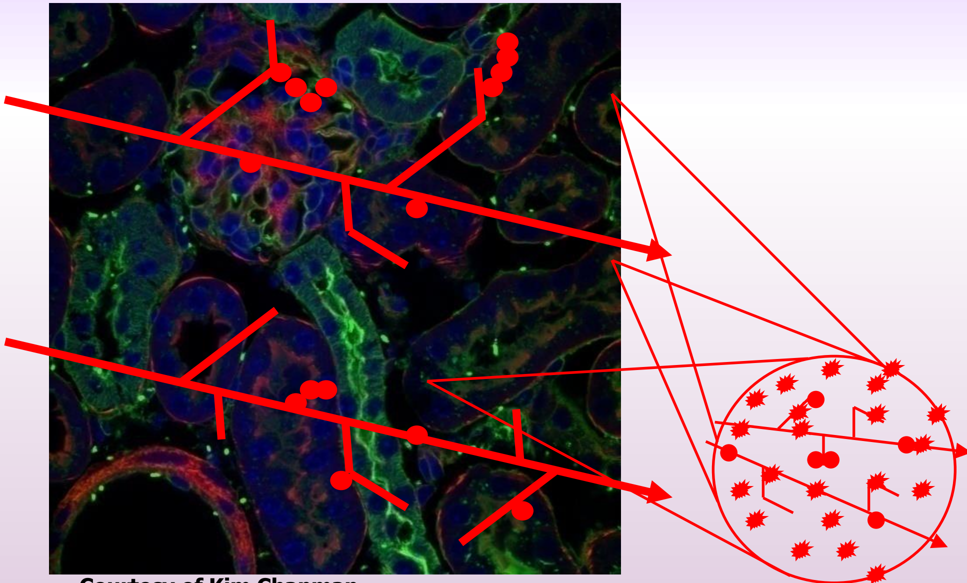
Penetrating powers of radiation can vary significantly for different types of radiation due to the <u>differences in their energy transfer properties</u>:

High LET (Alpha): Heavy, short-range particles; will not be able to penetrate human skin

Low LET (x, β & Gamma): Highly penetrating, Travels many inches through human skin

How does ionizing radiation interact within the cell/tissue?: It is Radiation Type dependent

Low-LET (e.g. γ-rays)

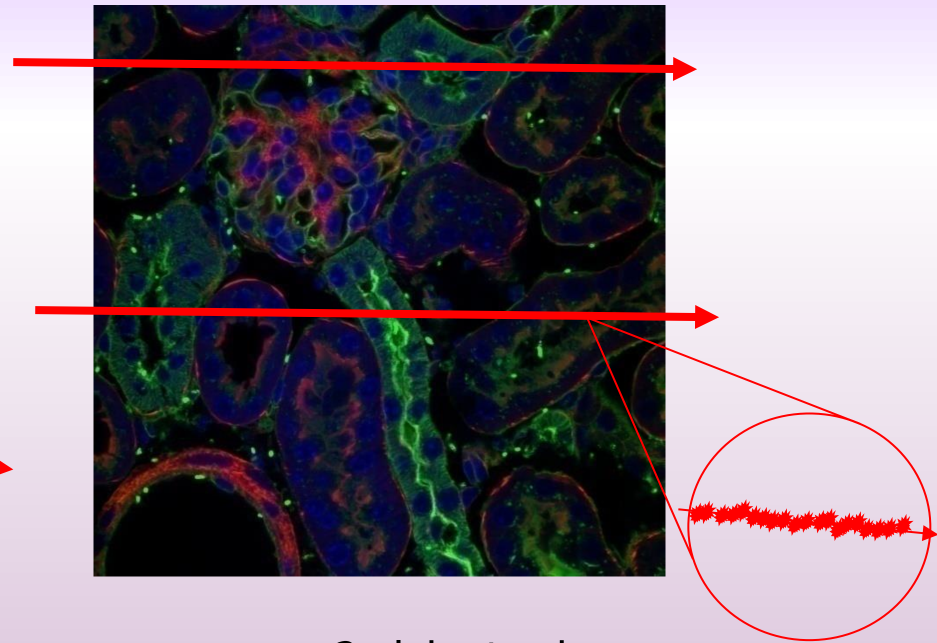


Courtesy of Kim Chapman

~1000 electron tracks

Relatively homogeneous

High-LET (e.g. α-particles)



~2 alpha tracks

Non-homogeneous

1 Gy corresponds to:

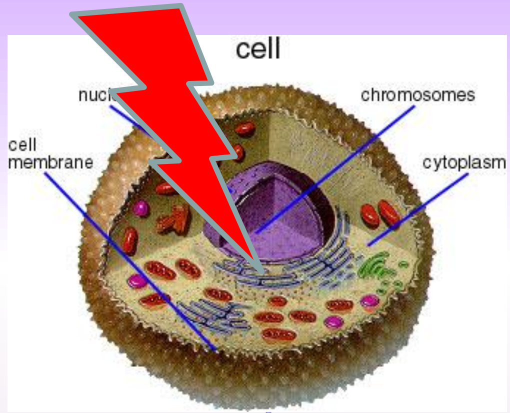
<u>Low LET</u> deposits the energy uniformly as detected by gamma H2X foci for DSB, produced randomly across the nucleus, while <u>high LET</u> is densely track , produce multiple DSB in the cells along the path of the particle / track.

Cellular events following radiation: two paradigms

- **The Ttarget / Classic Paradigm**: cellular responses is due to energy deposition in nuclear DNA
- **Non-Targeted Paradigm**: cellular responses that do not require direct exposure of the cell OR the cell's nucleus by radiation.

Cellular events following radiation: DNA damage recognition and response - The classical paradigm / the target paradigm

Radiation



Targeted Cell

DNA damage

High fidelity DNA repair

Senescence

Death

Radiation-induced Mutation(s) and /or chromosome rearrangements; leads to

Somatic

Germline

Foetal

Cancer

Heritable genetic effects

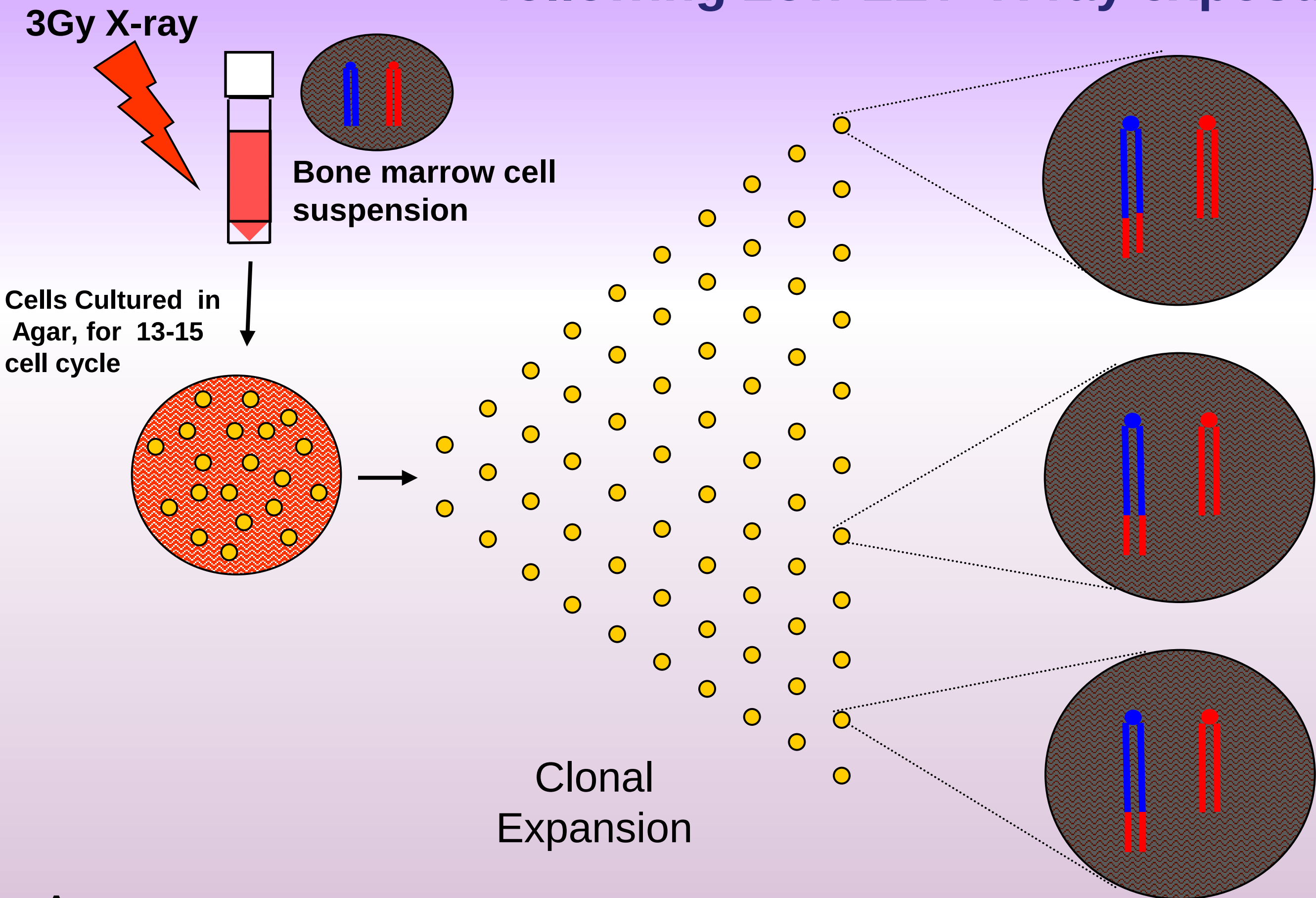
Developmental effects

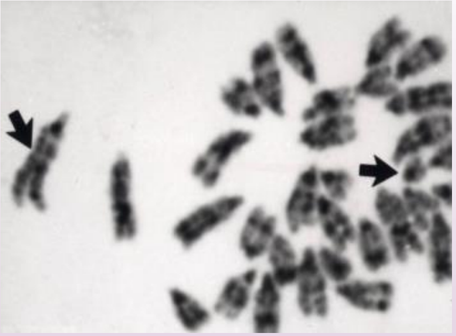
According to this paradigm, DNA is the target and deposition of energy is required in the responding cell

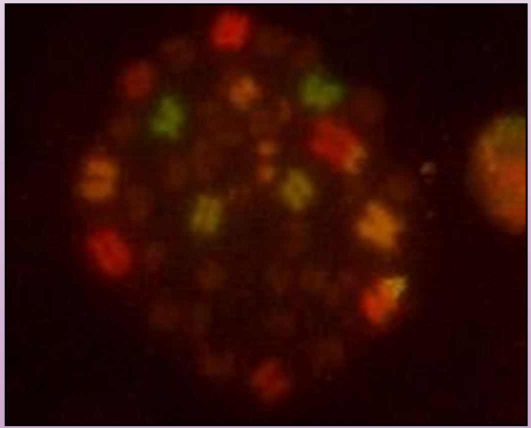
&

Most of these changes take place during the first cell/second cycle post-irradiation, fixed and transmitted to the cells progeny

Example : Clonal Chromosomal Aberrations in murine Haemopoietic cells following Low LET- X-ray exposure







Karyotypic Homoogeneity

Clonal Abberation

Arrows:
t (2;17)(q;qter), + frag(2)(cen)

Low frequency, clonal expression

Kadhim *et al.*, 1992. Nature 355, 738-740

Evolving Paradigm

Non-Targeted Effects of Radiation Exposure

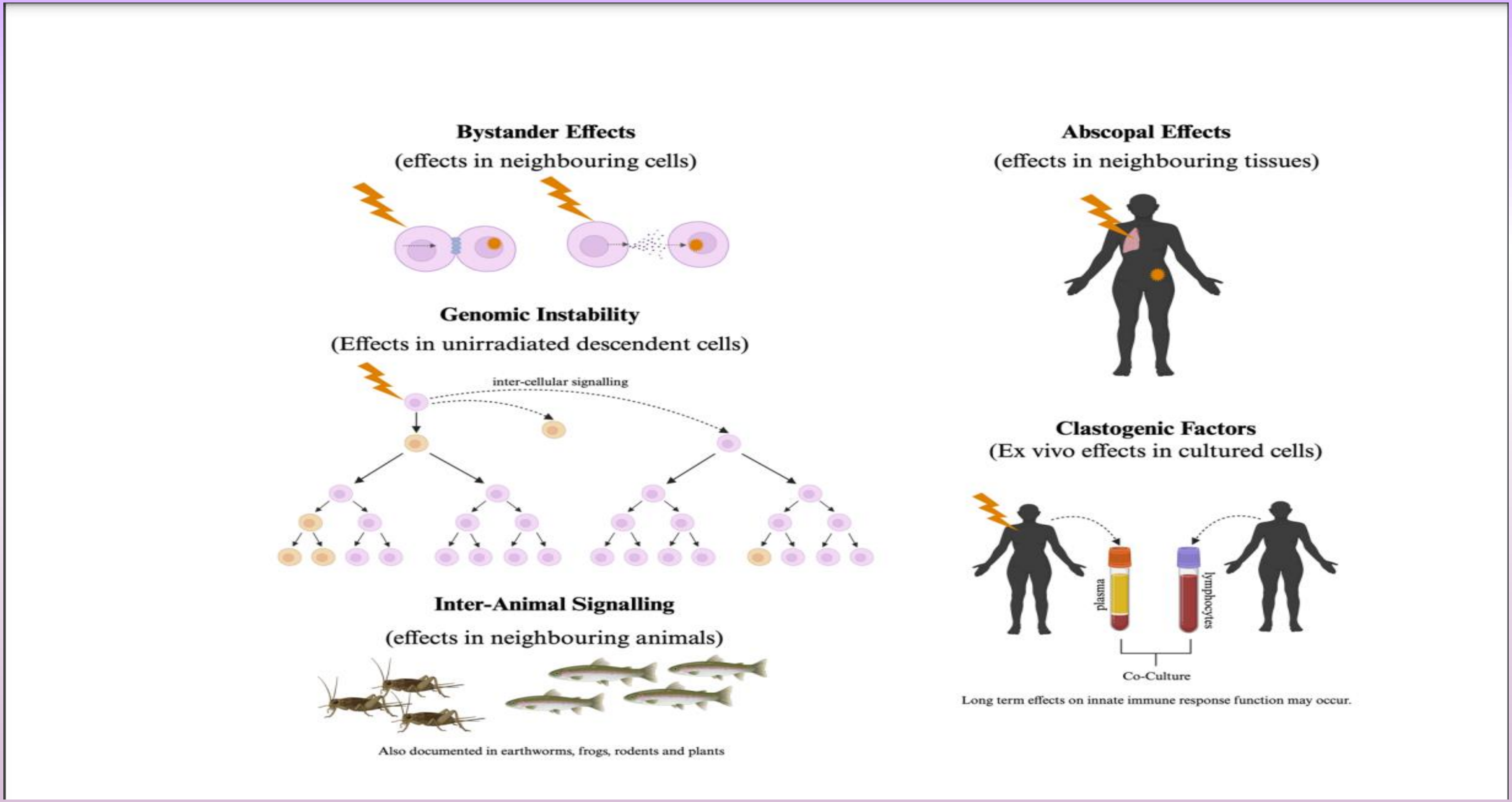
However,

Since the early 1990's evidence from numerous research findings show that the effects of exposure to ionizing radiation are not entirely attributed to the direct targeting of DNA & started to point to more complex effects:

"Non-targeted effects of exposure to ionizing radiation"

Reviews: ***Kadhim et al, 2013 ; Kadhim and Hill 2015; Julie J Burtt et al, 2016 ; Bright & Kadhim, 2018 , Rasoul Yahyapour et al, 2018, Venkatachalam Perumal, et al, 2018, Mothersill, et al, Cancers , 2019, Munira Kadhim et al, IJRB 2022,*** **X. He et al. BBA - General Subjects 1867 (2023) 130386 ;** ***Lyng & Azzamc; RADIATION RESEARCH 202, (2024)***)

Various non-targeted effects



- Fiona M. Lyng, Edouard I. Azzamc, Abscopal Effects, Clastogenic Effects and Bystander Effects: 70 Years of Non-Targeted Effects of Radiation *RADIATION RESEARCH 202, 000–000 (2024).*
- Carmel Mothersill, at all, 2017 , - History of bystander effects research 1905-present; what is in a name? *INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, 2017 ;* *https://doi.org/10.1080/09553002.2017.1398436*
- E Daguenet et al., Radiation-induced bystander and abscopal effects: important lessons from preclinical models; British Journal of Cancer (2020) 123:339–348; *https://doi.org/10.1038/s41416-020-0942-3 BJC,*
- Radiation-Induced Rescue Effect: Insights from Microbeam Experiments. Kwan Ngok Yu, Biology **2022**, 11, 1548. *https://doi.org/10.3390/biology11111548*

Courtesy of Dr. Carmel Mothersill

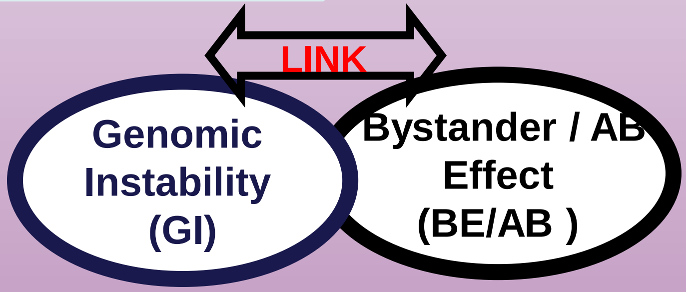
Non-Targeted Effects of Radiation Exposure Continue ….multiple responses - include :

Genomic Instability (GI)

Genetic changes detected in the unirradiated descendants/ progeny of the cells which were originally irradiated & survived

Bystander/ Abscopal Effects

Induction of biological effects in neighbouring cells/ tissues that are not directly irradiated through cell-cell communication.



The Original Study which leads to Non-Targeted Effect of Radiation Exposure paradigm

Gardner's Report

BMJ VOLUME 300 17 FEBRUARY 1990

Results of case-control study of leukaemia and lymphoma among young people near Sellafield nuclear plant in West Cumbria.

Martin J Gardner, Michael P Snee, Andrew J Hall, Caroline A Powell, Susan Downes, John D Terrell

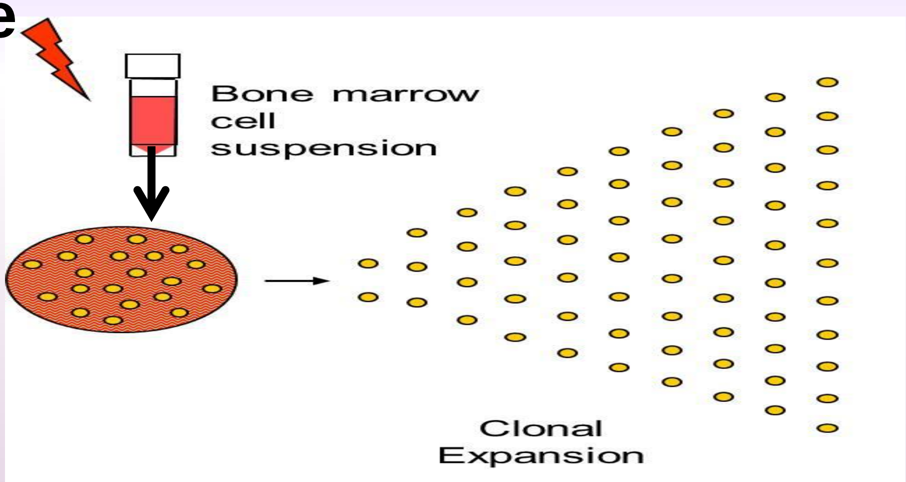
The conclusion of this report suggested that :

The increased incidence of leukaemia, particularly, non-Hodgkin's lymphoma among children near Sellafield was associated with paternal employment at the plant before conception & suggests an effect of ionising radiation on fathers that may be leukaemogenic in their offspring.

This report lead to the investigation into:
"Radiation-Induced NTE "Genomic Instability (GI)".

High LET α-particle effects on Haemopoietic stem cells

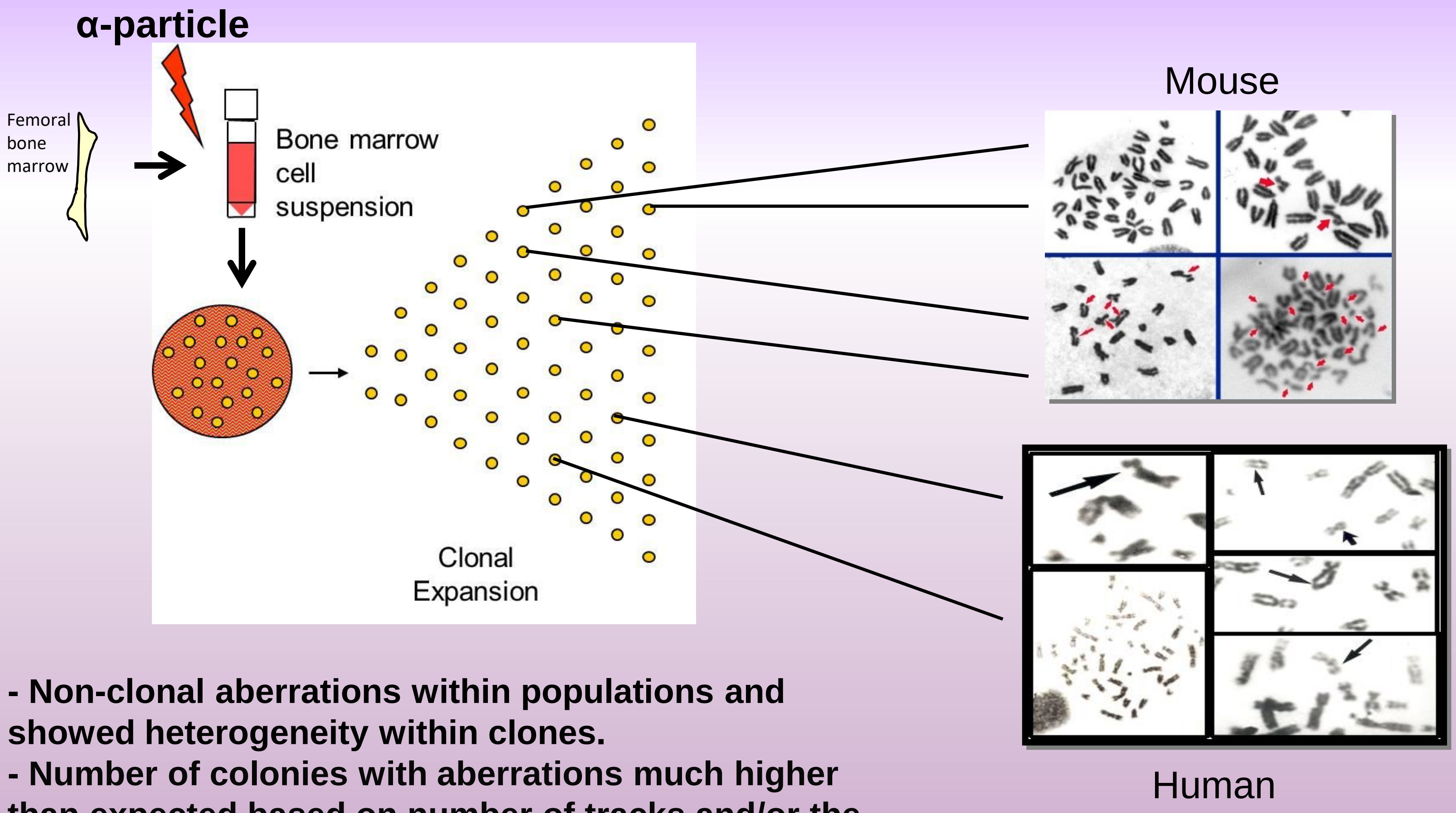
α-particle



Similar Experimental System (Murine Haemopoietic cells) **with High LET α-particle exposure** but different & un-expected results were observed.

(Kadhim *et al.*, 1992, Nature 355, 738-740 ; Kadhim *et al*, 1994, Lancet 344, 987-988)

Example: Non-clonal chromosomal aberrations/instability in descendants of irradiated haemopoietic stem cells

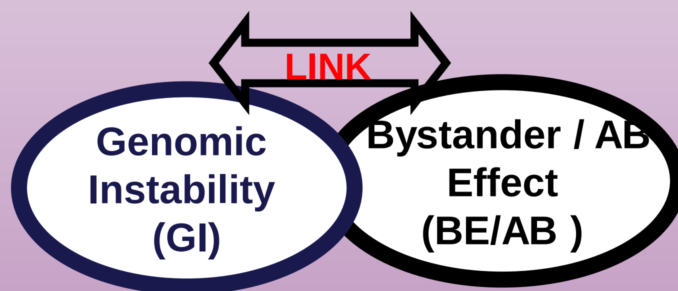


- Non-clonal aberrations within populations and showed heterogeneity within clones.
- Number of colonies with aberrations much higher than expected based on number of tracks and/or the frequency of survival cells ,suggesting : <u>chromosomal damage instability in the non-hit bystander cells (Bystander Effects).</u>

Kadhim *et.al*., 1992, *Nature*, 355, 738
Kadhim *et.al*., *1994, Lancet*, 344, 987

Non-Targeted Effects of Radiation Exposure: multiple responses - include :

Genomic Instability (GI)	Bystander/ Abscopal Effects
Genetic changes detected in the descendants of the cells that were originally irradiated & survived	Induction of biological effects in neighbouring cells/ tissues that are not directly irradiated but through molecules cell-cell communication



Radiation induced BE under different exposure scenarios. Irradiated (red) cells can communicate with each other or with unirradiated cells (white).

Bystander Effects

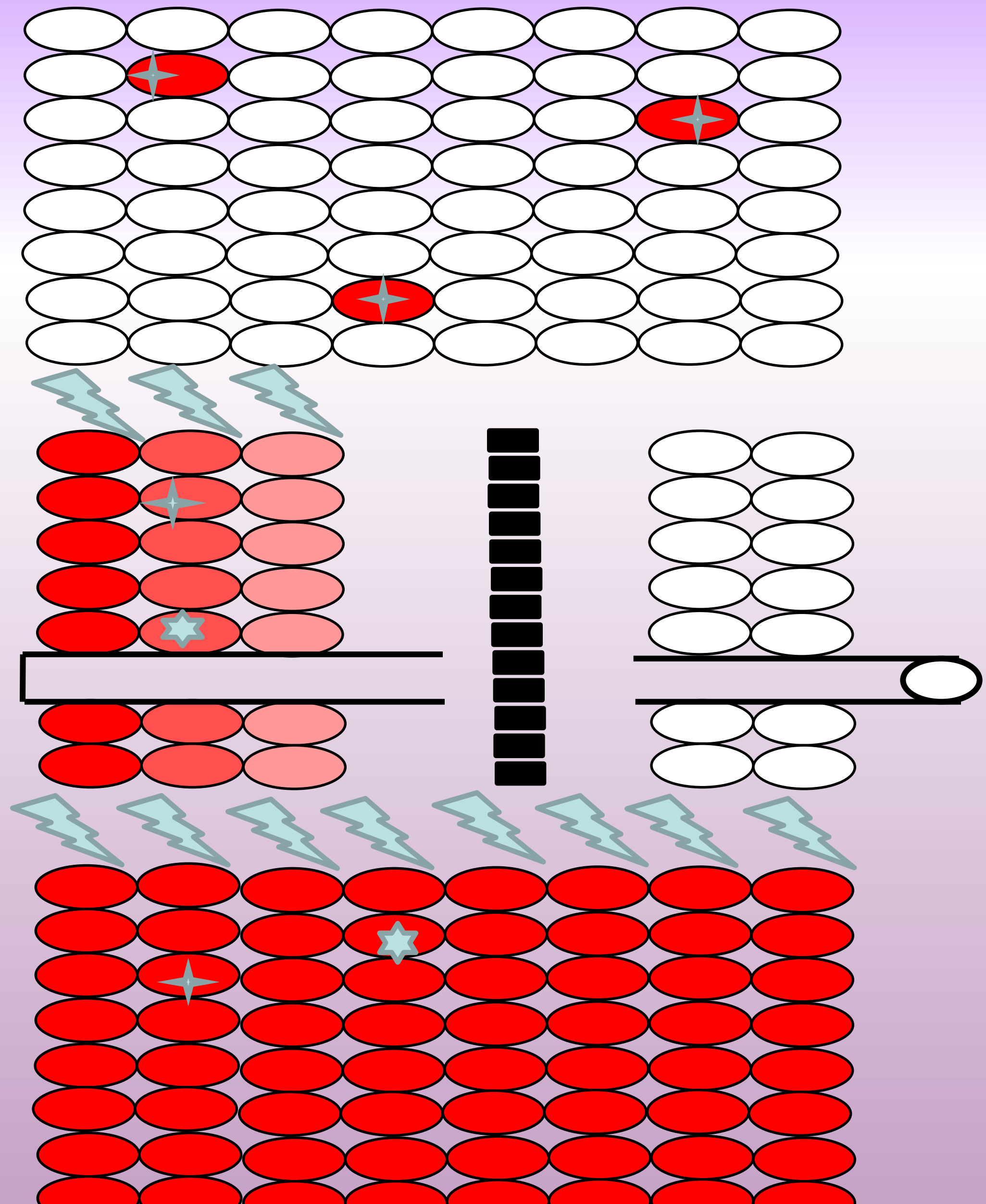
Effects are due to communication between irradiated cells and nearby unirradiated cells

Abscopal Effects

Effects are due to communication between irradiated tissues and unirradiated tissues outside of the irradiated field, via systemic signalling

Cohort Effects

Effects occur between irradiated cells within an irradiated tissue



Adapted from Blyth and Sykes (2011) Radiat. Res 176, 139.

Evidence for radiation induced bystander effects *In vitro* & *In vivo*

In vitro

•Increased levels of Chromosomal Instability in haemopoietic stem cells (Kadhim et al, *Nature*,1992) and SCE in CHO cells irradiated with low doses of α-particles (Nagasawa and Little, *Cancer Res,* 1992).

• Extracellular factors involved in SCE following α-particle exposure (Lehnert and Goodwin, *Cancer Res,* 1997).

• Medium from γ-rays irradiated cells reduces the survival of unirradiated cells (Review: Mothersill and Seymour, *Radiat Res,* 2001).

• Bystander effect after microbeam irradiation of a single cell (Prise *et al*, *IJRB*, 1998; Belyakov *et al, BJC,* 2001) or a fractions of cells (Moore et al, *Radiat Res,2005).*

• Induction of a bystnader mutagenic effect after α-particle microbeam irradiation (Zhou *et al, PNAS.* 2000).

• Bystander effect and genomic instability under *in vitro* (Lorimore *et al, PNAS,* 1998) and *in vivo* conditions (Watson *et al, Cancer Res,* 2000).

• A proliferation-dependent bystander effect inurotheial explants after microbeam irradiation (Belyakov*et al, BJC,* 2003).

•Y Zhao, S M de Toledo, G Hu, T K Hei and E I Azzam (2014). Connexins and cyclooxygenase-2 crosstalkin the expression of radiation-induced bystander effects. British Journal of Cancer 111, 125–131

•Ziqi Zhang et al , 2022, Radiation-Induced Bystander Effect and Cytoplasmic Irradiation Studies with Microbeams; Biology 2022, 11, 945. https://doi.org/10.3390/biology11070945

In vivo

•Studies in rat lung models have shown evidence for cytokine driven responses leading to damage in non-irradiated parts of the lung (Khan *et al.,*1998, *Int J Radiat Oncol Biol Phys,* 40, 467)
•Radioisotope studies in mouse models have shown evidence for bystander responses (Xue *et al.,* 2002, *PNAS,* 99, 13765)
•Close relationship between instability and bystander responses in mouse models (Lorimore *et al.,* 2005, *Cancer Res,* 65, 5668
•Increased apoptosis levels detected in the cerebellum in shielded head animals -suggesting significant contribution from bystander signals (Mancuso *et al.* (2008 ,*PNAS* 105(34): 12445-12450).
•Radiation-induced bystander and abscopal effects: important lessons from preclinical models Elisabeth Daguenet et al, 2020 , British Journal of Cancer (2020) 123:339–348; https://doi.org/10.1038/s41416-020-0942-3
•Abscopal effects are observed clinically after radiotherapy (Kaminski *et al.,*2005, *Cancer Treat Rev,* 31, 159)
•***"Abscopal effect – a biological effect of radiation that occurs at a distance from the irradiated tissue"*** (Mole, 1953, *Br J. Radiol.,* 26, 234. Whole body irradiation: radiobiology or medicine?).

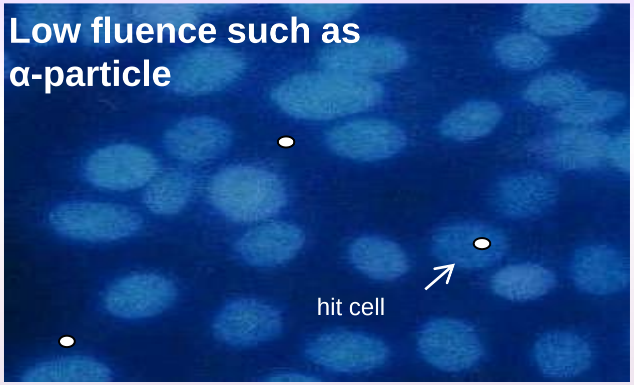
Bystander Effects (BE)

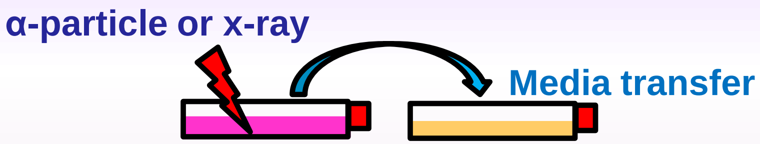
Bystander effects are not unique to ionising radiation.

- **UV biophotons 2006 (discovered),** 2007–2017, Mosse et al. (2006); Mothersill et al. (2007a); Ahmad et al. (2013); Le et al. (2015a, 2015b): First physical factor proven to transmit bystander signals; **UV** & BE (Whiteside and McMillan, 2009; Dahle *et al.*, 2005)
- **The Bystander Effect of UV and Mediators**, (Eftekhari and Fardid, 2020)
- **Photosensitisers** (Charkroborty *et al.*, 2009)
- **Heat** (Purschke *et al.*, 2010)
- **Chemoagents** (Mitomycin (Asure *et al*., 2010)
- **Gene therapy agents** (Mesnil *et al.*, 1996)
- **Radiotherapy Outcomes, Virgínea de Araújo Farias, et al , 2020 (Review), 2020**
- **Chemical** (Rajalakshmi, et al, 2009; Asur, *et al, 2010*);
- **HIV-1 Virus (**Himanshu, *et al* , 2012)
- **Oncolytic viruses** impact bystander cells, (Leslee Sprague, et al, 2020)
- **Activation of bystander CD8+ T** cells and their roles in viral infection (Kim and Shin, 2019)
- **History of bystander effects research 1905-present; what is in a name**?

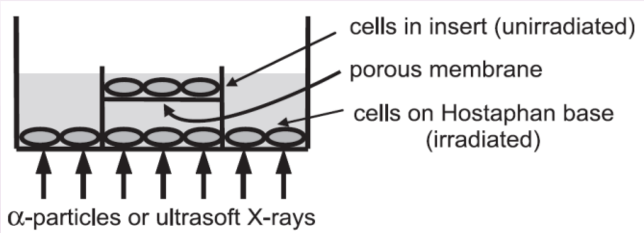
  Carmel Mothersill, et all, 2017; International Journal of Radiation Biology, 2017.

Experimental techniques used to study Bystander effects include:

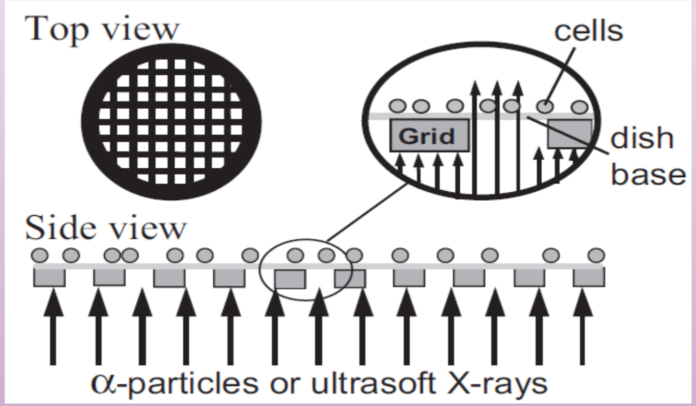




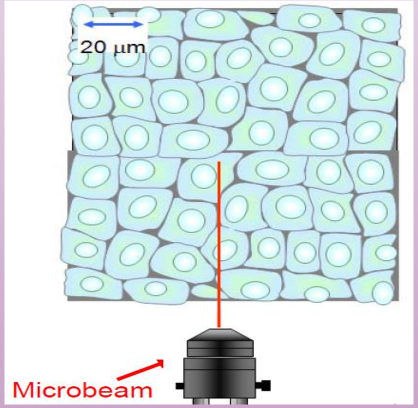
Co-culture



Partial shielding



Microbeams are facilities that allow irradiation of individual cells or cell regions with precise numbers of charged particles with *micrometer* precision.



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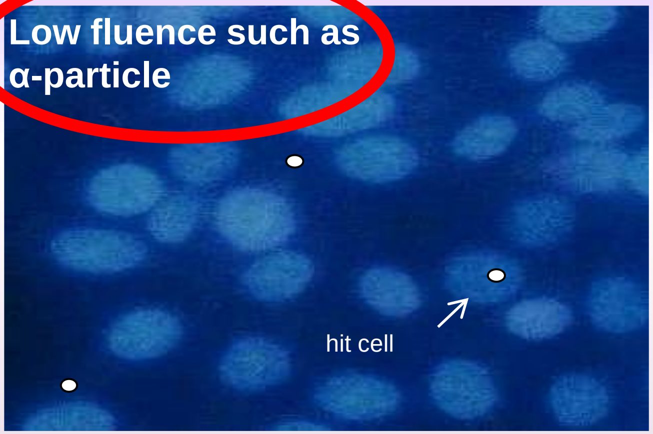

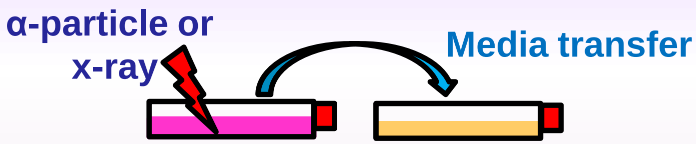

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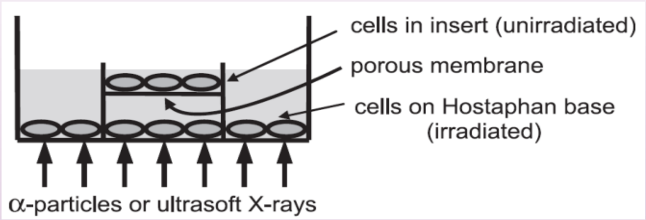

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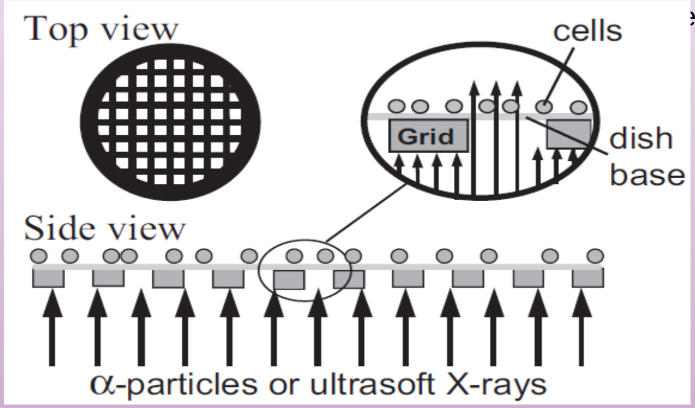

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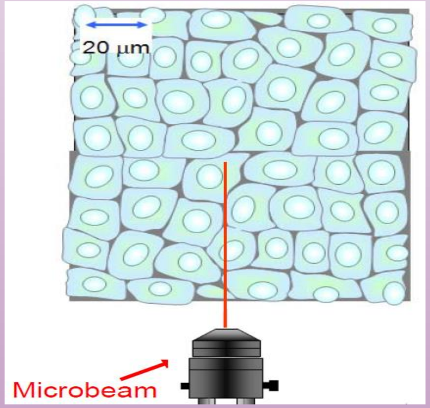
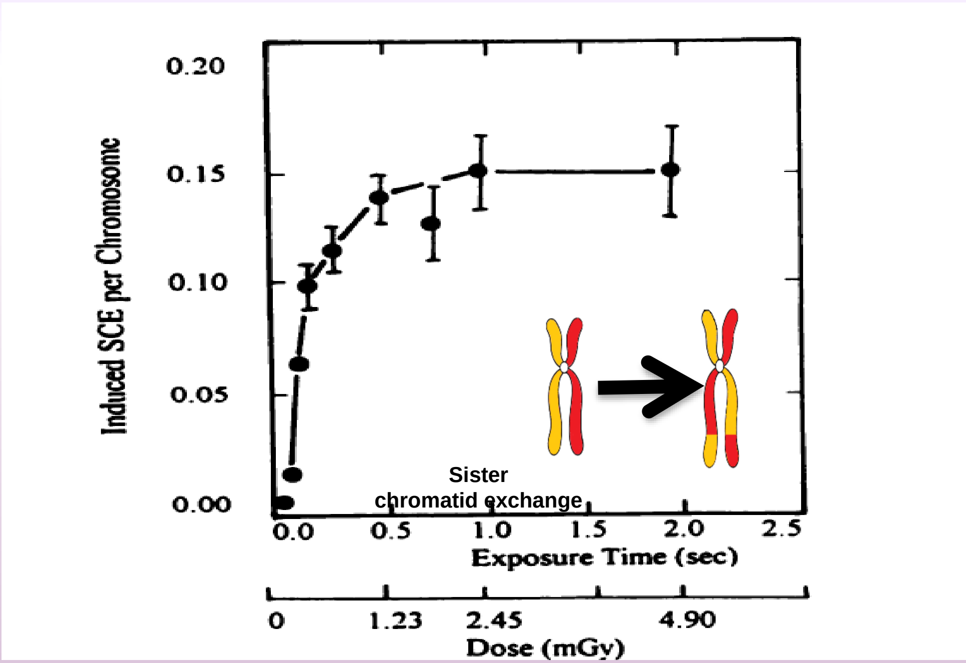


Bystander Effects after Low Fluences of α -Particle Irradiation

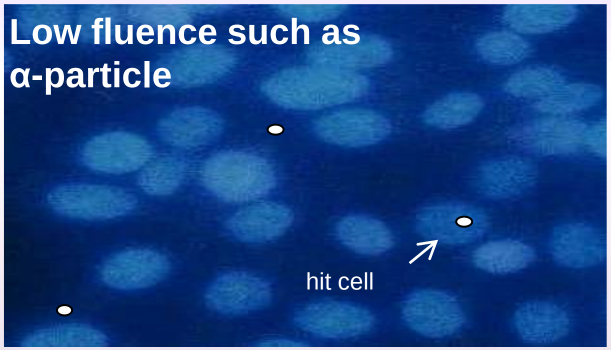
Sister chromatid exchange **frequency increases in 30% of cells even though 1% cells traversed**



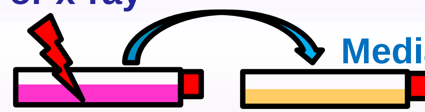
Nagasawa & Little 1992

The results showed the inducing chromosomal damage by α-particle was much larger than the nucleus or cell itself.

Experimental techniques used to study Bystander effects include:

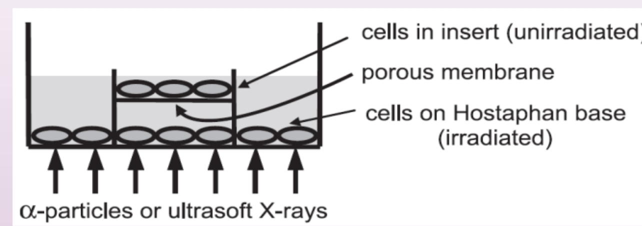


α-particle or x-ray



Media transfer

[Co-culture]



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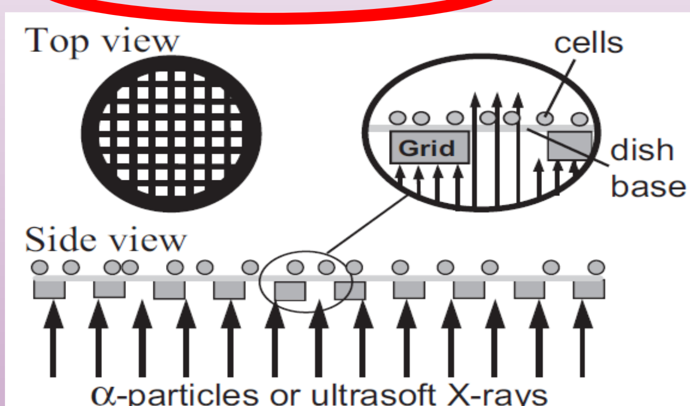

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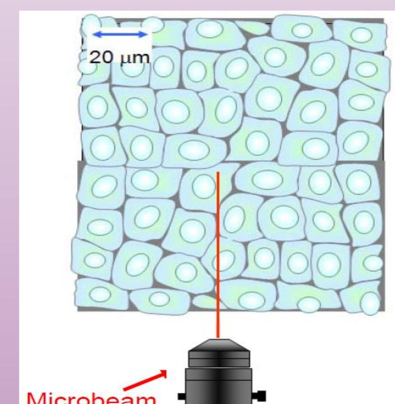
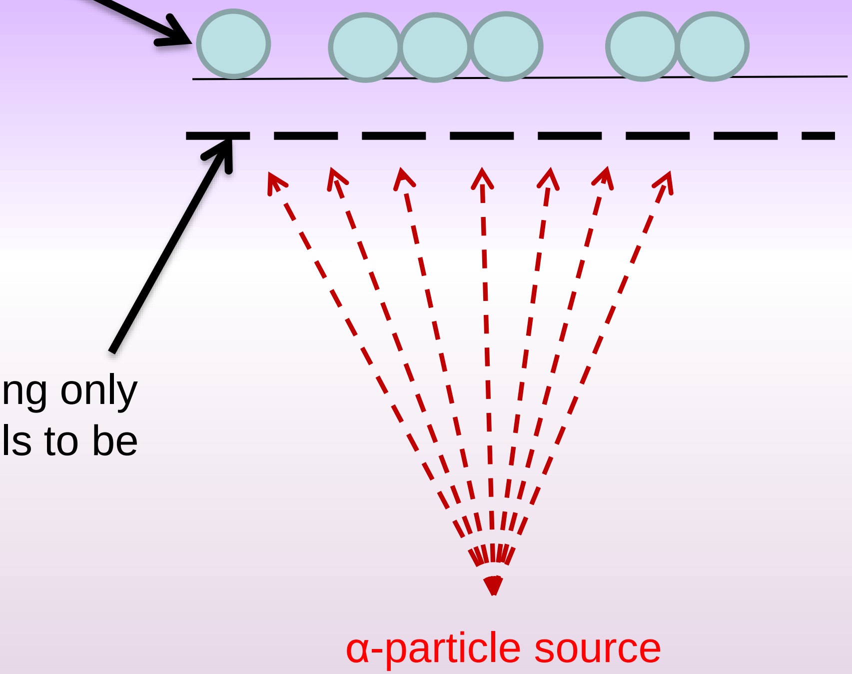
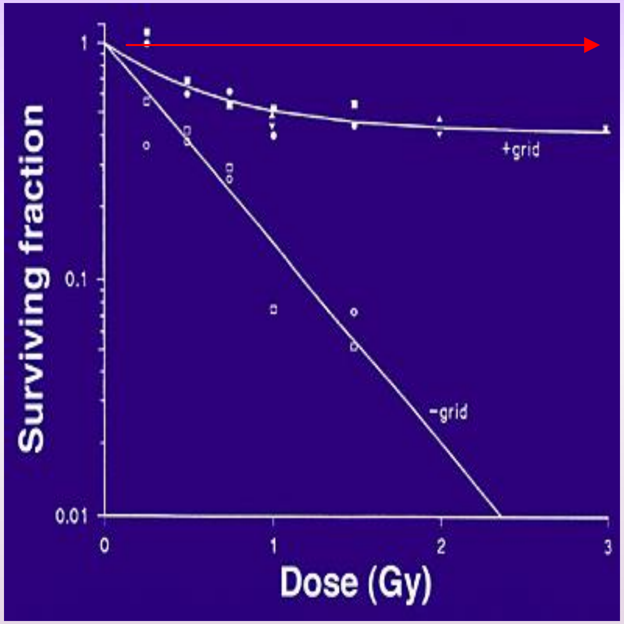


Partial shielding: Bystander and genomic instability – Linked

Mouse haemopoietic steam cells



Grid allowing only 50% of cells to be irradiated



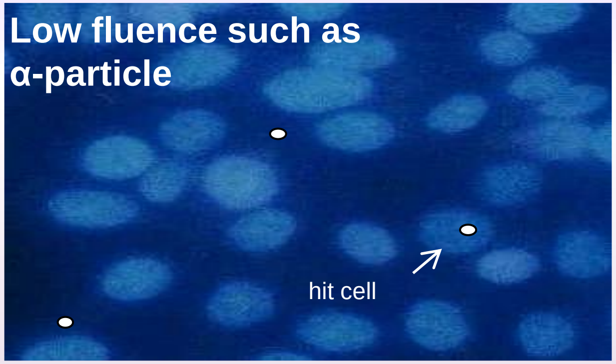
No evidence for bystander cell killing

α-particle irradiation dose (Gy)	Surviving fraction	Mean number of aberrations per cells (%)
Control	1.00	7.0
1Gy - grid	0.01	22.0
1Gy + grid	0.58	21.0

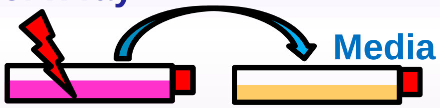
The level of instability is similar despite the presence of the grid shielding 50% of targeted cells

Lorimore, SA, Kadhim, MA, Pocock, DA, Papworth, D, Stevens, DL, Goodhead, DT, Wright, EG (1998), *Proceedings of the National Academy of Sciences* **95:**5730-5733.

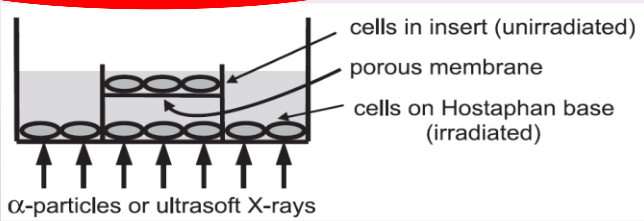
Experimental techniques used to study Bystander effects include:



α-particle or x-ray



Co-culture



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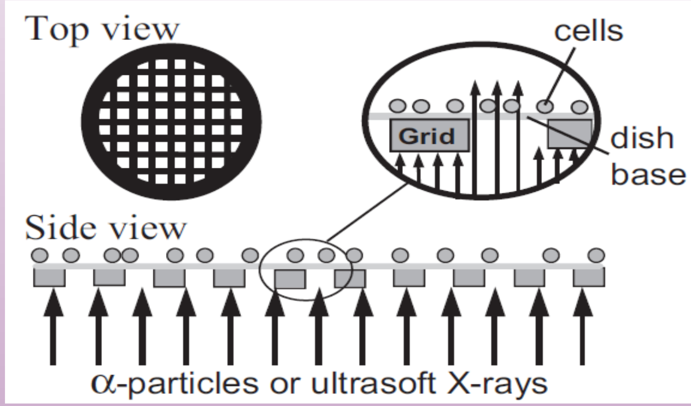

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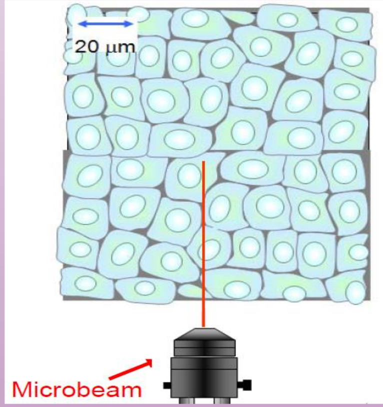

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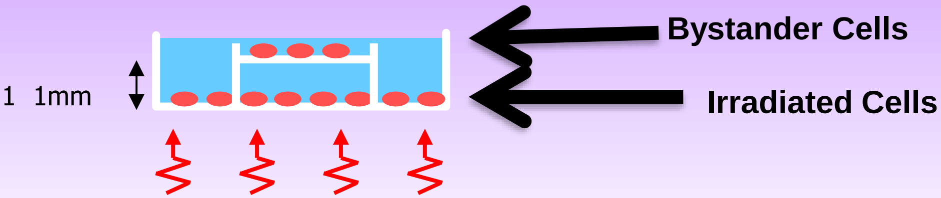
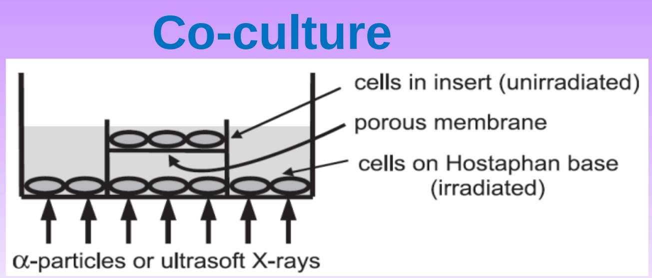
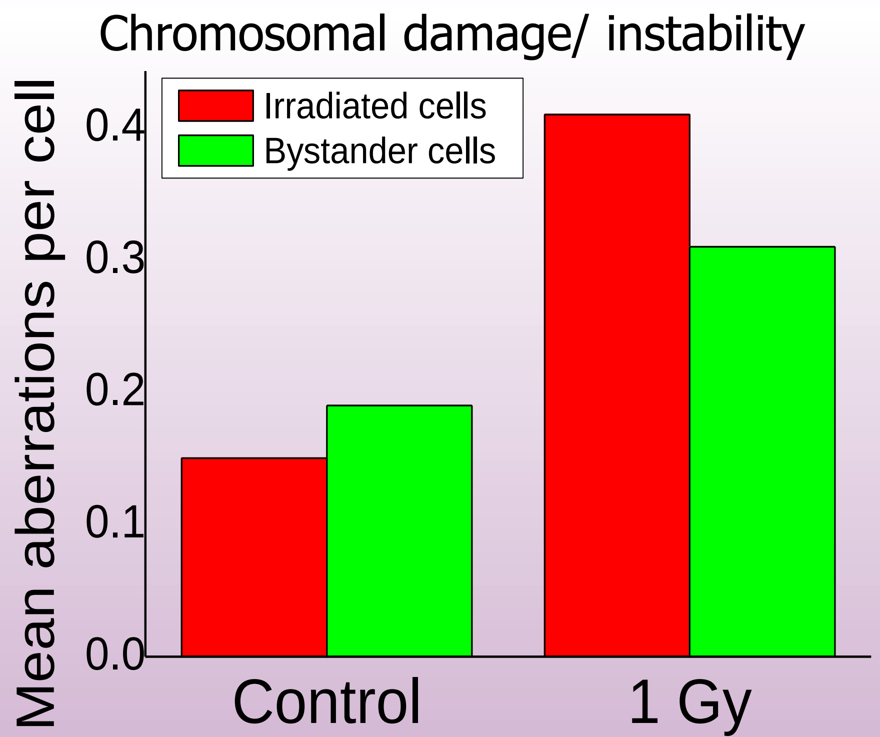
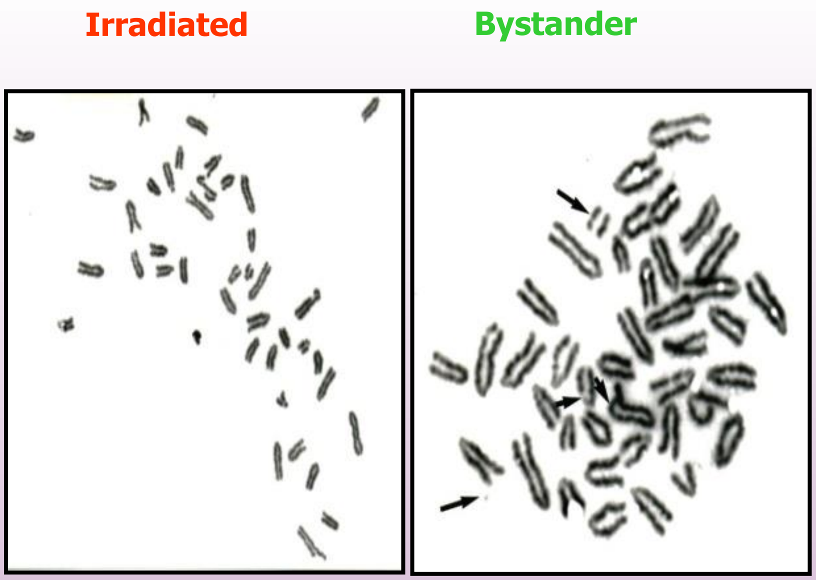




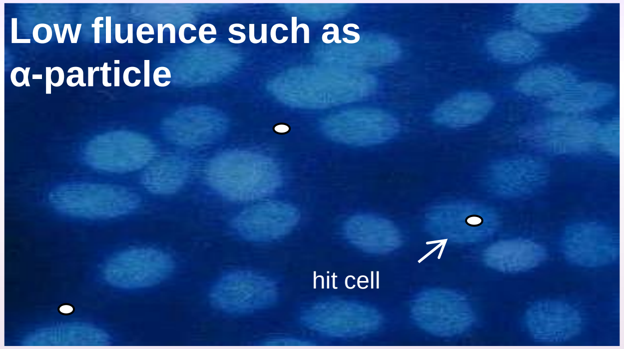




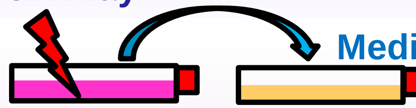
Similar types (quality) of chromosomal aberrations in the irradiated and bystander population

Bowler *et al*, 2006

Experimental techniques used to study Bystander effects include:

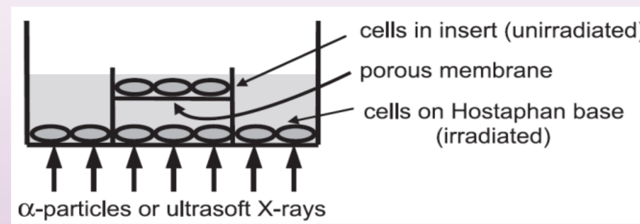


α-particle or x-ray

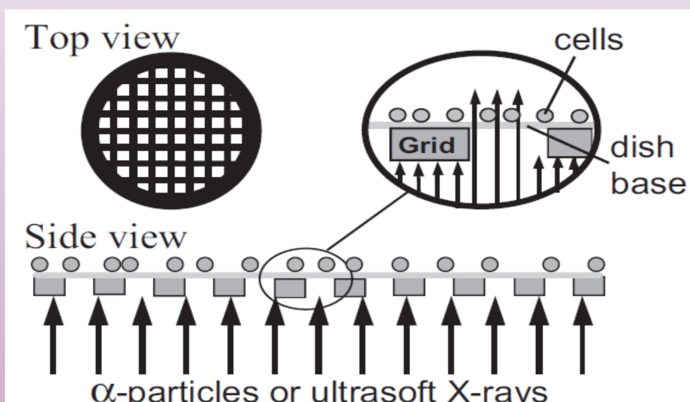


Media transfer

Co-culture



Partial shielding



ects

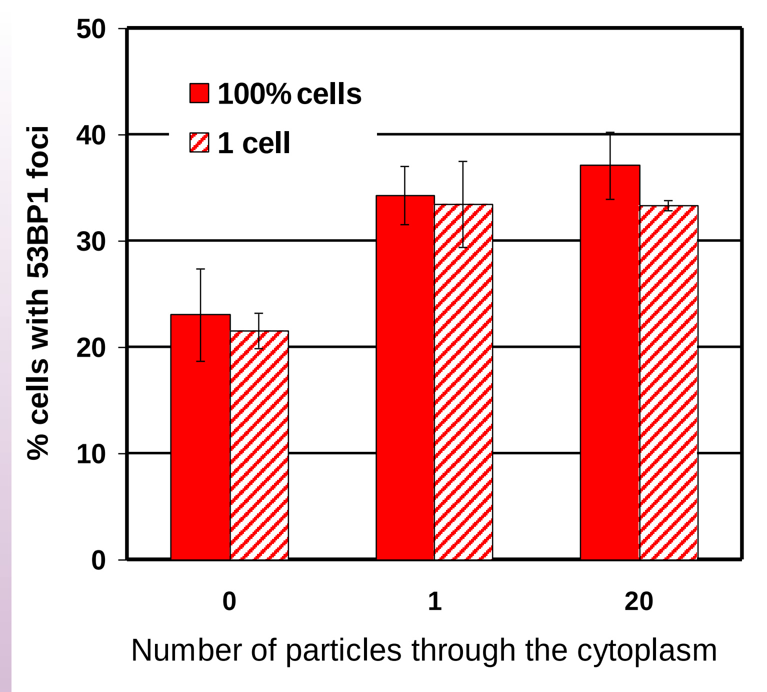
Microbeams

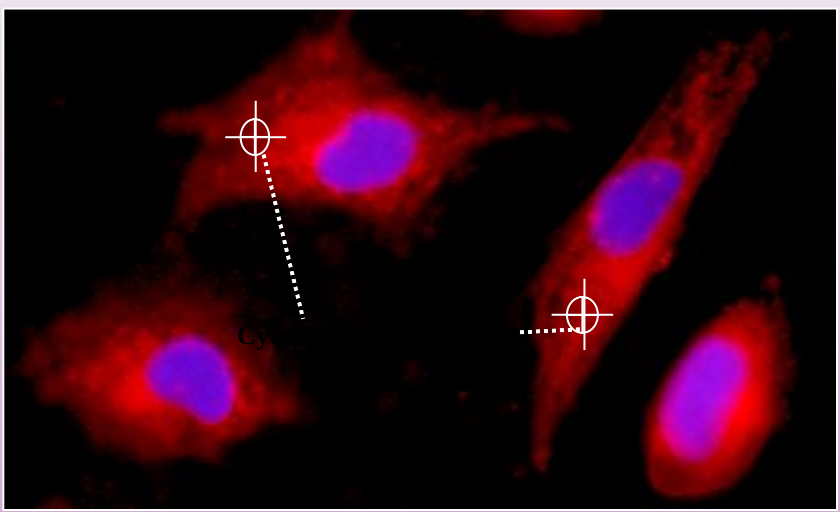
are facilities that allow irradiation of individual cells or cell regions with precise numbers of charged particles with *micrometer* precision.

Targets and Bystanders

- To observe a bystander effect, the DNA does not have to be directly irradiated

Cytoplasmic targeting





1 or 20 Helium ions

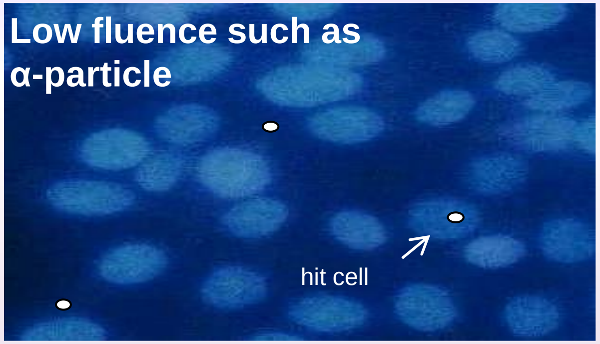
3 hours

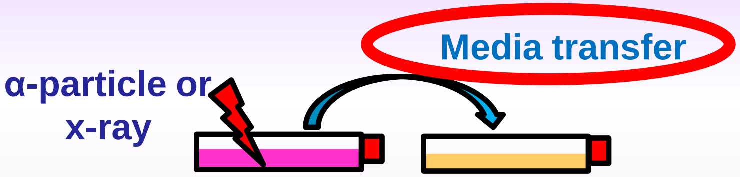
Direct effect

Bystander effect

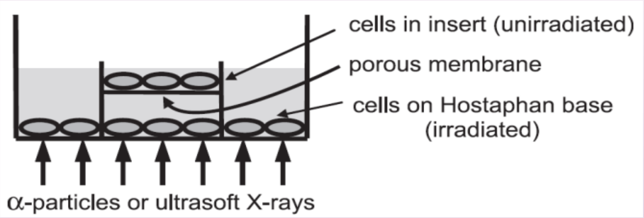
Tartier *et al.*, 2007, *Cancer Res.*, **67,** 5872

Experimental techniques used to study Bystander effects include:





Co-culture



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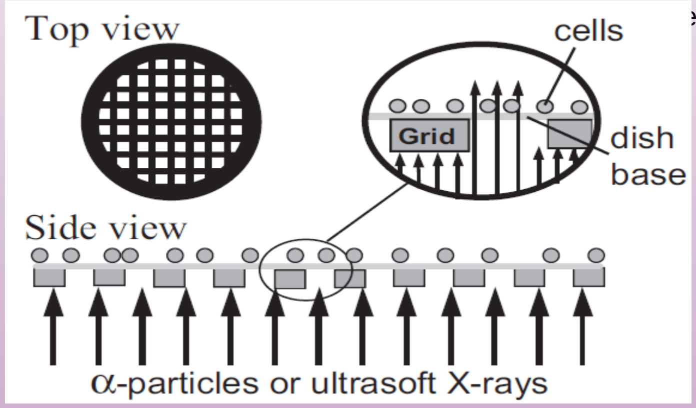

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Microbeams

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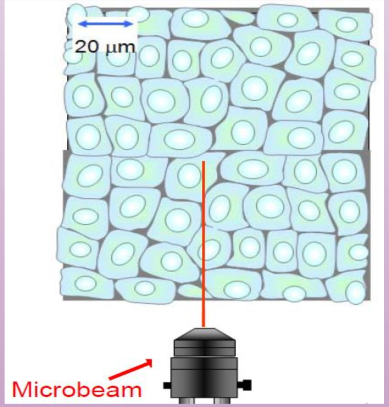
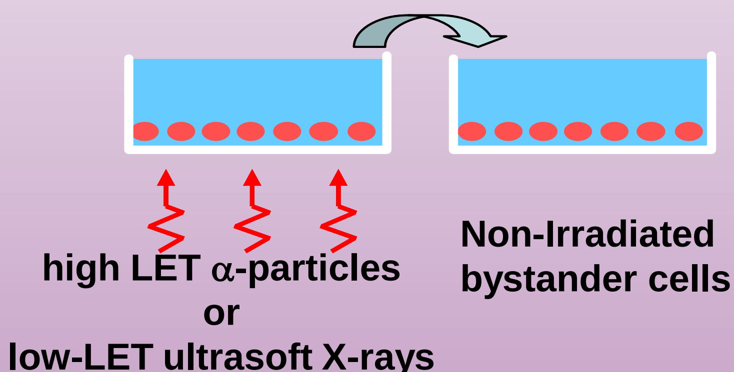
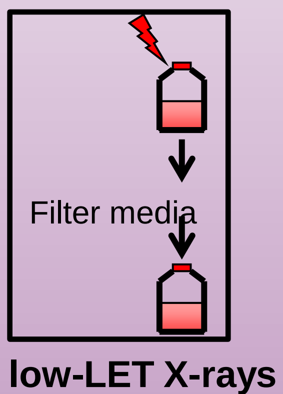


Media Transfer Method

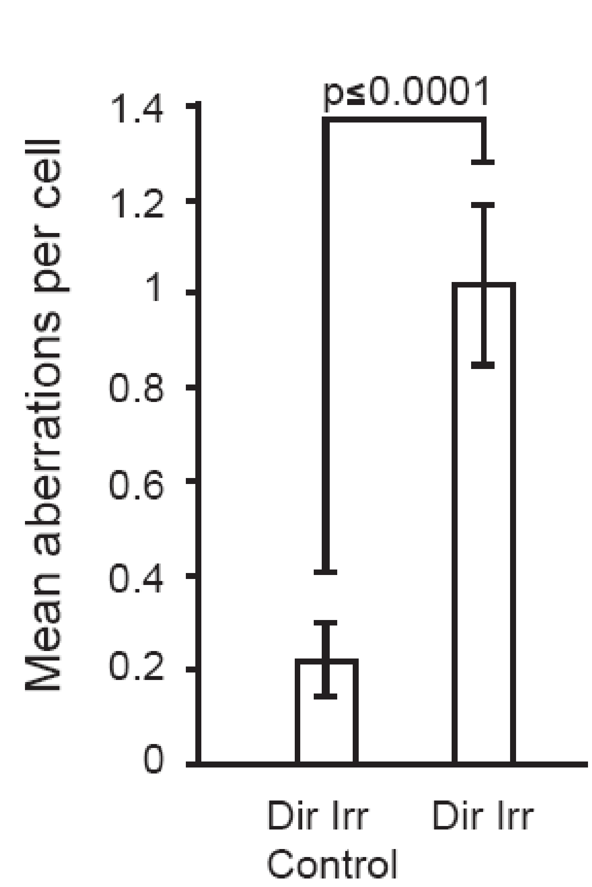
<u>Bystander Effects after Transfer of Medium from Irradiated Cells</u>

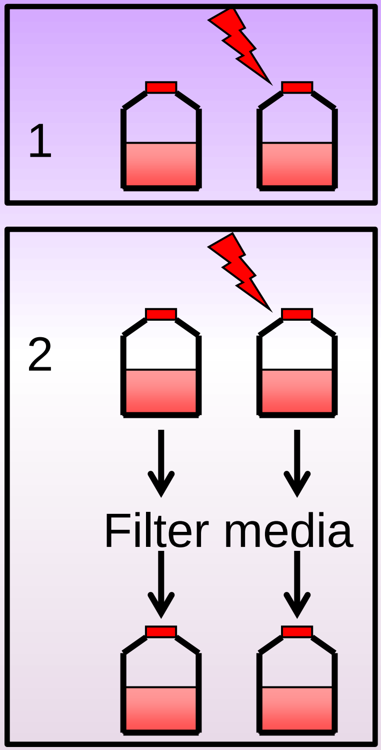
Medium transfer approach is used for investigating Bystander Effects of both High and Low LET radiations *in vitro* and *in vivo* (Review: Mothersill and Seymour, *Radiat Res*, 2001).





Media transfer

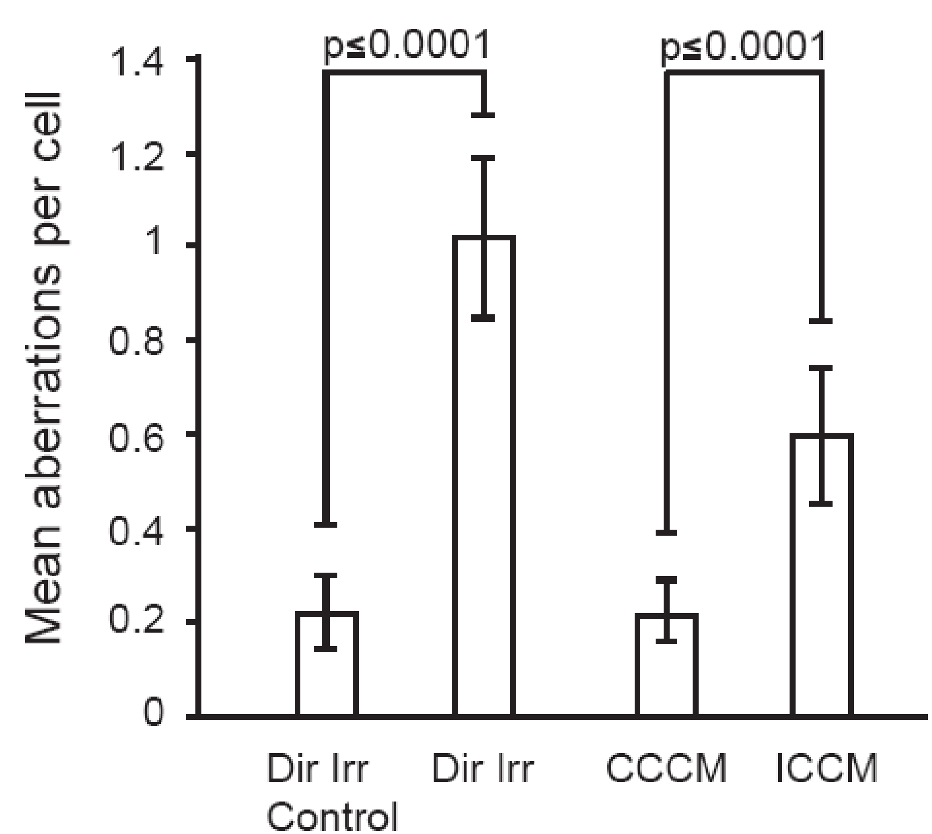




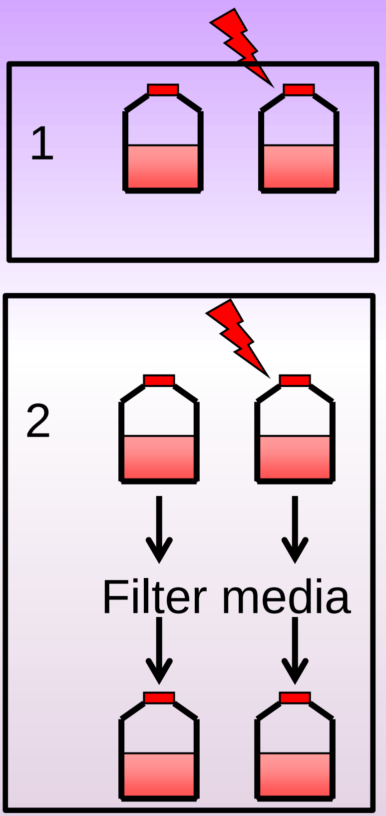
1

Media transfer

BYSTANDER



1 2



Bystander Effects *in vivo*

In ***vivo*** studies

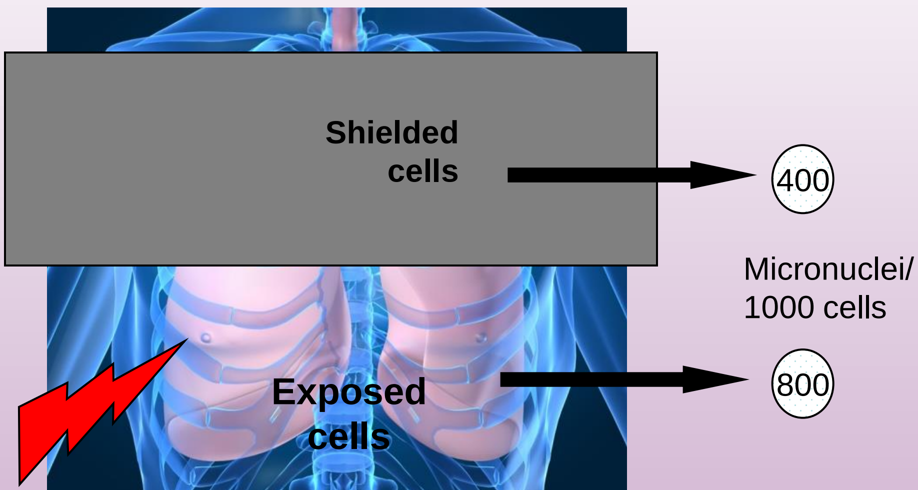
- Bystander effects in an *in vivo* human skin model (3D)
  - Belyakov *et al*. PNAS 102, 14203-7 (2005)
- Studies in rat lung models have shown evidence for cytokine driven responses leading to damage in non-irradiated parts of the lung
  - Khan *et al.*, (1998), *Int J Radiat Oncol Biol Phys,* **40,** 467
- Radioisotope studies in mouse models have shown evidence for bystander responses
  - Xue *et al.*, (2002), *PNAS*, **99,** 13765
- Close relationship between instability and bystander responses in mouse models
  - Lorimore *et al.*, (2005),*Cancer Res*, **65,** 5668
- Increased apoptosis levels detected in the cerebellum in shielded head animals -suggesting significant contribution from bystander signals
  - Mancuso *et al.* (2008) ,*PNAS* **105**(34): 12445-12450

- Abscopal effects are observed clinically after radiotherapy
  - Kaminski *et al.*,*(*2005), *Cancer Treat Rev*, **31,** 159
- ***"Abscopal effect – a biological effect of radiation that occurs at a distance from the irradiated tissue"***
  - Mole, 1953, *Br J. Radiol.*, **26,** 234. Whole body irradiation: radiobiology or medicine?

– Radiation-induced bystander and abscopal effects: important lessons from preclinical model , Daguenet et al, 2020 , British Journal of Cancer (2020) 123:339–348

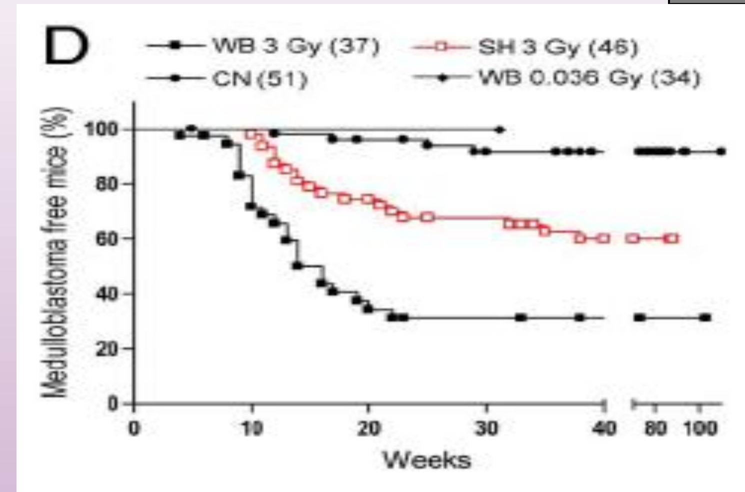
Bystander effects: *in vivo*

- **Lower half of lung exposed to 10Gy**
- **Upper lung cells shielded from direct irradiation show increased chromosomal damage**
- **Long range communication within the lung-cytokine involvement**



Redrawn from Khan *et al., IJRB* (1998)

- **Head shielded, body irradiated (3Gy X-ray)**
- **Medulloblastoma observed in 39% of head-shielded mice (~80% previously observed for whole body irradiation)**
- **Increased apoptosis levels in cerebellum of shielded cells.**



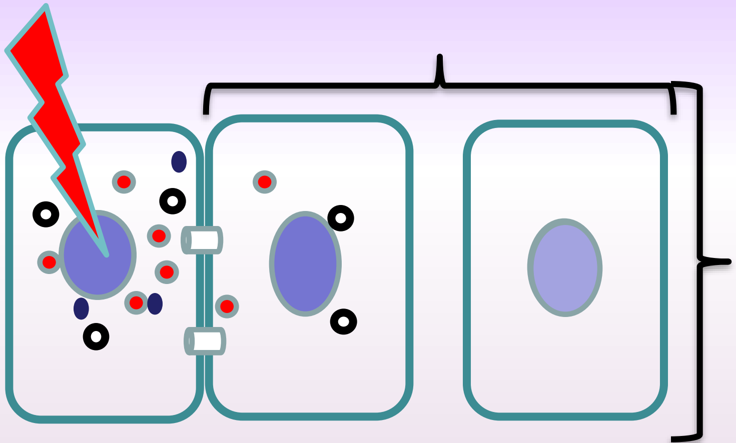
- **Evidence for manifestation of bystander radiation effects at the whole body level**
- **Deregulation of several miRNA were involved**

Mancuso *et al. PNAS* 2008; Simonetta Pazzaglia, et al,*Int. J. Mol. Sci. 2021*

POSSIBLE MECHANISMS

Mechanisms & Consequences of Ionizing Radiation-Induced Bystander Effect: intercellular communication can occur between neighbour cells via Gap Junction and/or Diffusion Factors

Irradiated cells generate and secrete stress communication signalling



Recipient bystander cells receive stress signalling

Direct irradiated cell

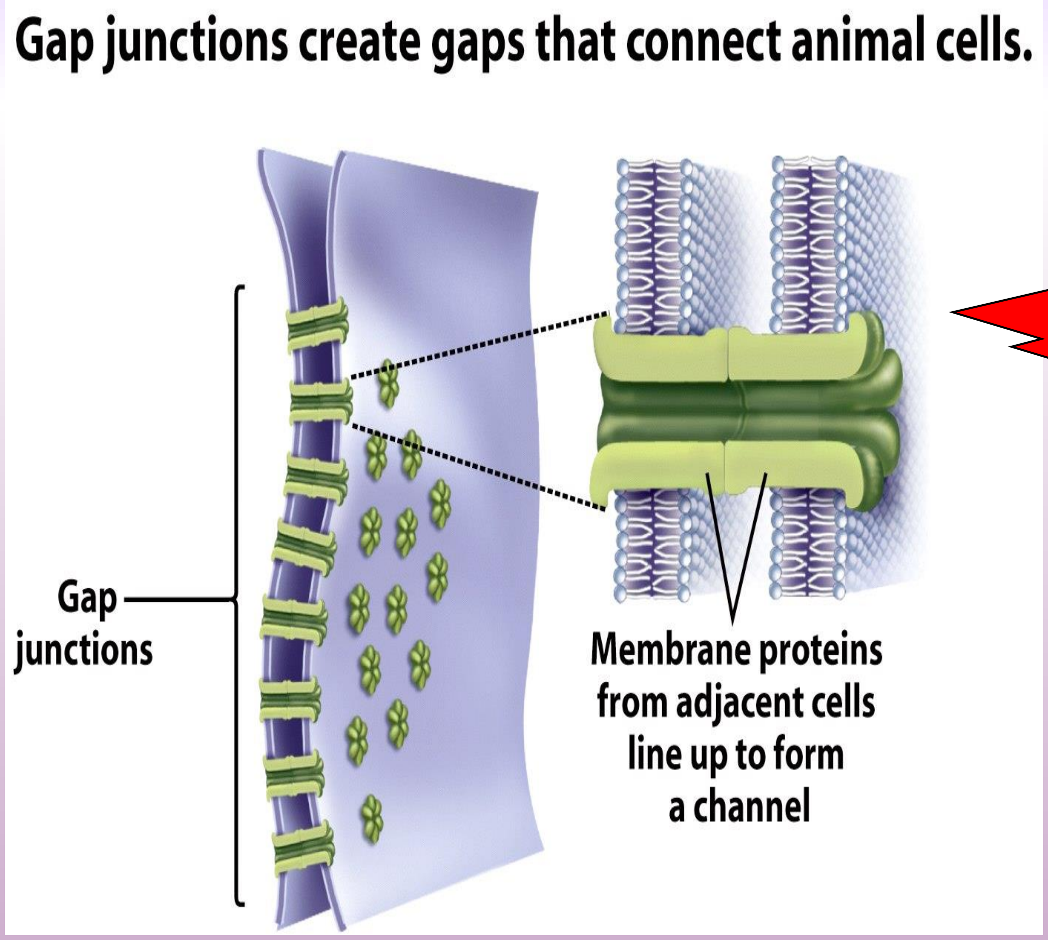
Bystander cell (**Gap junction**) Azzam et all, 2011& **others**

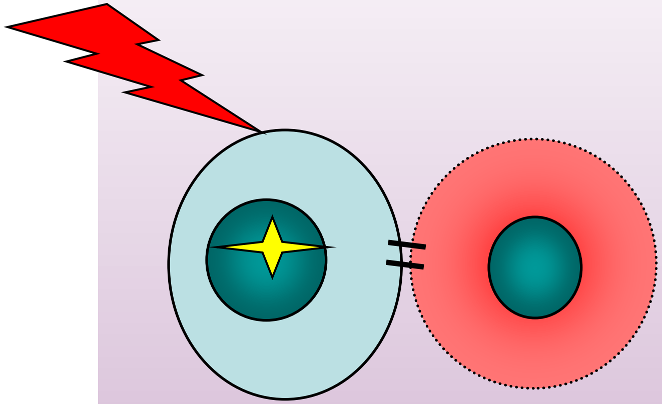
Bystander cell (**Diffusion factors**), Mothersill,1997, Al-Mayah 2012 **& others**

Responses to the signals include

Apoptosis, chromosomal instability , mutation, senescence, induction of stress and adaptive pathways and metastasis

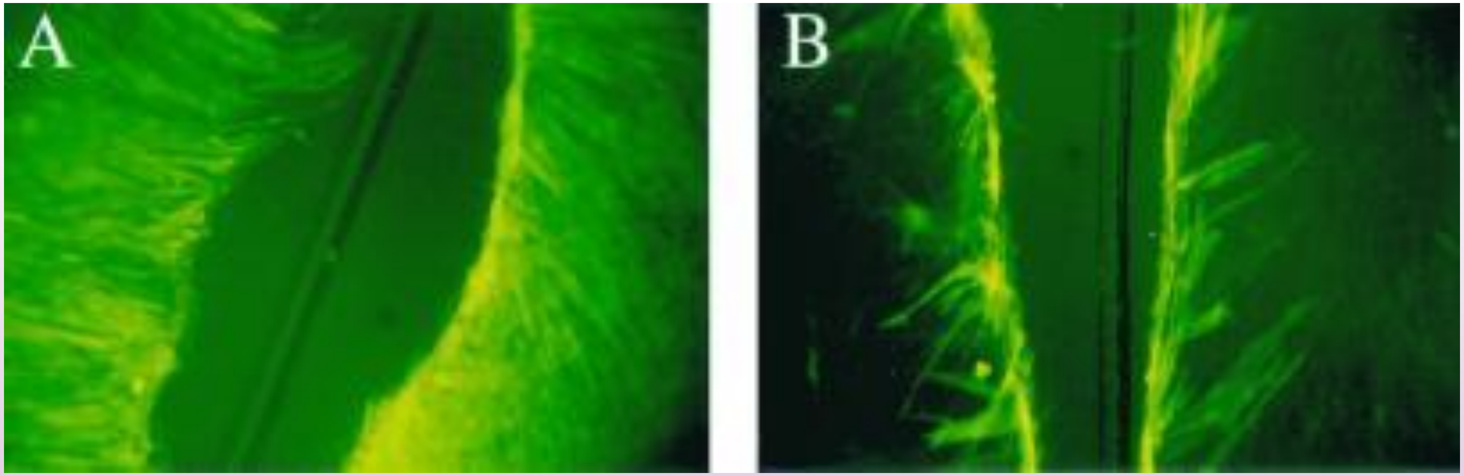
1-Gap Junction Communications





Direct cell-cell communication between adjacent cells via gap junctions was demonstrated (Azzam *et al,* 2001)
Connexin 43 (Cx43), the most widely expressed GJ protein

Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from α-particle irradiated to non irradiated cells



(A) Transfer of the fluorescent dye Lucifer yellow through gap junction in AG1522 confluent cultures, and (*B*) inhibition of its transfer to adjacent cells by 40 μM lindane.

Azzam *et al* (2001) Proc Natl Acad Sci U S A. 98(2): 473–478.

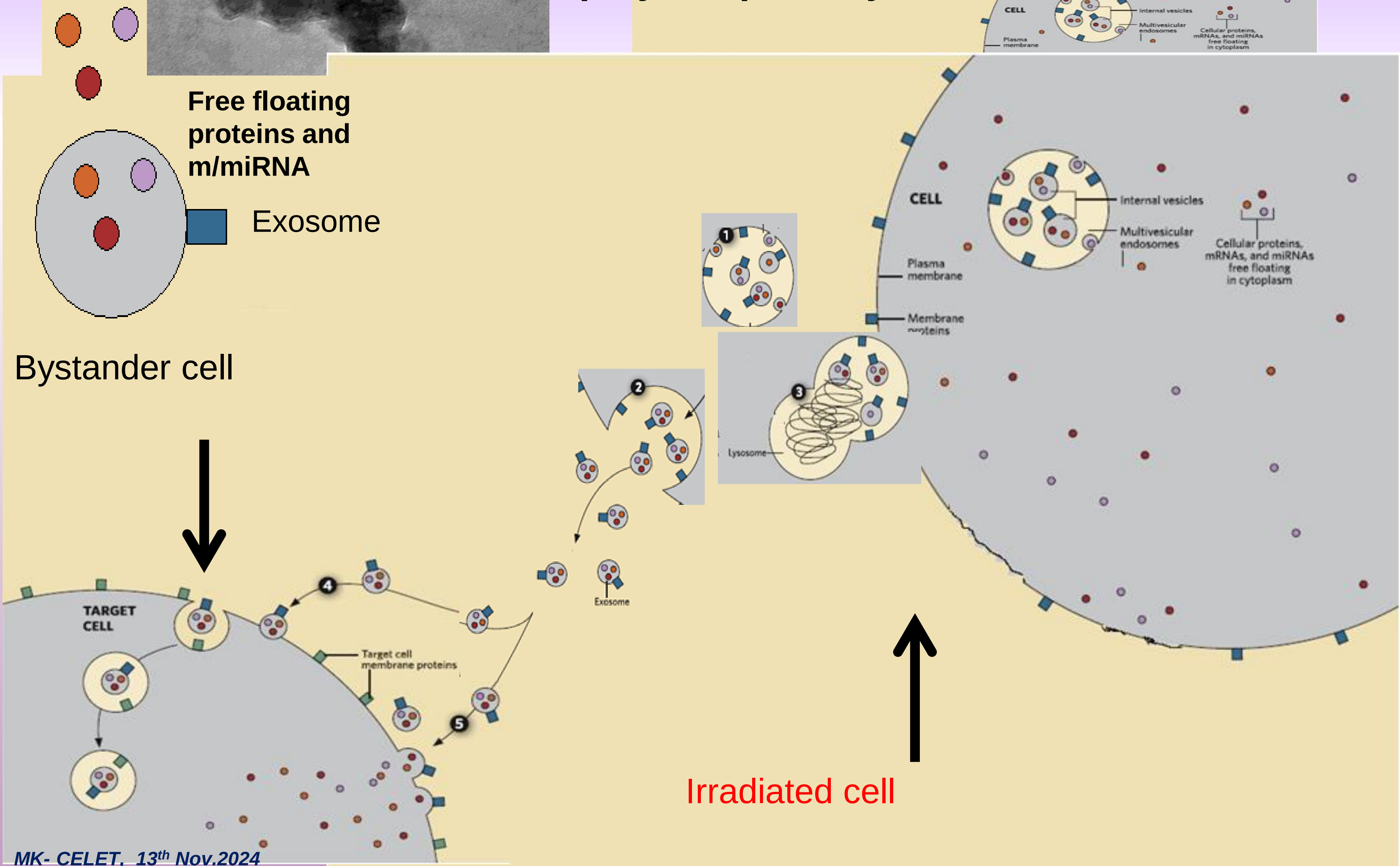
2- Role of Secreted Diffusible Factors / Signalling Molecules in Radiation-Induced Bystander / Abscopal Effects

2- Diffusion Factors Mediated :Bystander/ Abscopal Signals

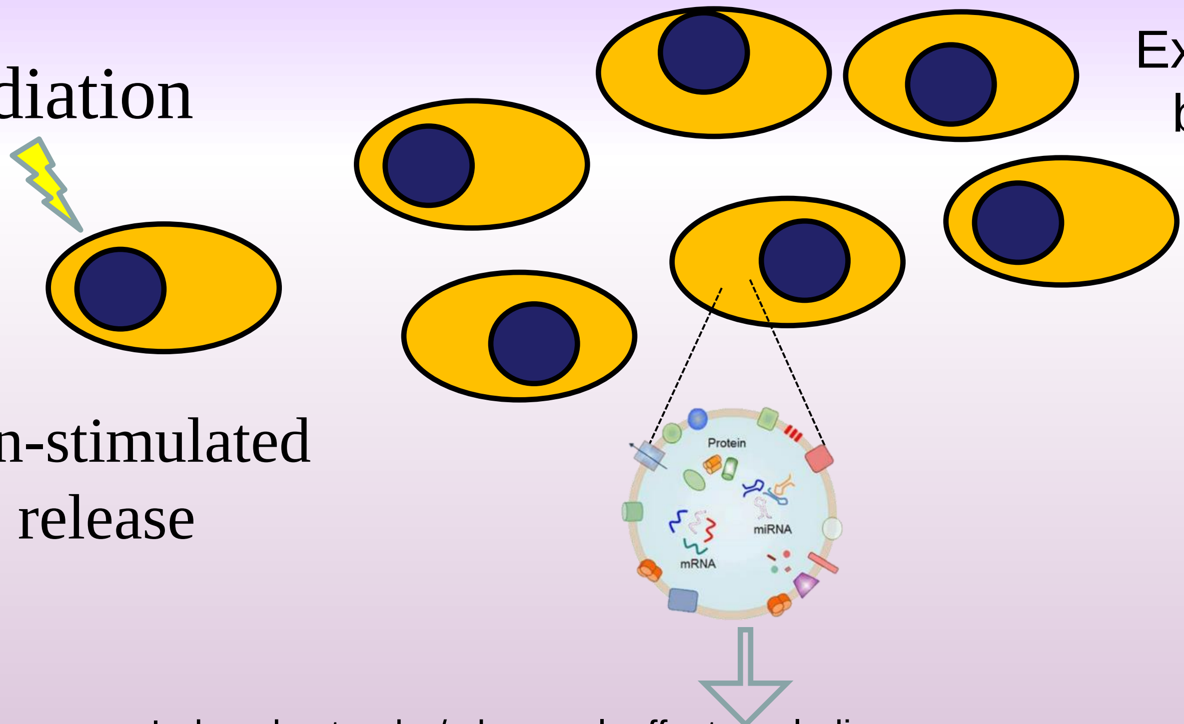
Communication mediators	Effect upon responses induction	Reference
ROS	Prevention of growth arrest	(Macip et al. 2002) (Jella et al. 2018)
NO	Activation of radioresistance among bystander cells	(Najafi et al , 2014) (Jella et al. 2018)
protein kinases	Protein kinase B and protein kinase C are involved in ROS production and oxidative damage in bystander Cells	(Blume-Jensen et al. 2001; Hunter et al. 2000)
miRNAs	Upregulation of miRNAs due to irradiation increases serum levels of them that affect the expression of target genes in non-irradiated tissues.	Prise et al. 2003; Chaudhry and Omaruddin 2012; Kadhim et al. 2013; Najafi et al. 2014)
Cytokines i.e. TNF-α	Reduction in radiation-induced apoptosis	(M. Zhang et al. 2008)
Mitochondria	Reduced γ-H2AX induction	(Chen et al. 2008)
Gap-junctions	Reduced p53 modulation/reduced mutagenesis	(Zhou et al. 2001; Azzam et al. 1998) (Autsavapromporn et al, 2013)
COX-2	Reduced DNA damage	(Zhou et al. 2005 ; Zhao et al. 2014)
Calcium	Prevention of micronuclei induction	(Shao et al. 2006b)
Extracellular vesicles/ **Exosomes**	Abrogation of DNA damage mediation via an RNA/ Protein dependent mechanism	(Al-Mayah et al. 2012,2015, 2017; Jella et al, 2014; Mo et al. 2018; Tuncay Cagatay,et al 2020; Kadhim et al.2021) Simonetta P.et al,2022, Raheem AL-Abedi, *Int. J. Mol. Sci.* 2021, 22, 11

EXOSOMES

Secreted from irradiated cells to the extra cellular environment & can be taken up by recipient bystander cells



Role of Secreted Diffusible Factors / Signalling molecules in Radiation Induced Bystander Effects (BE)

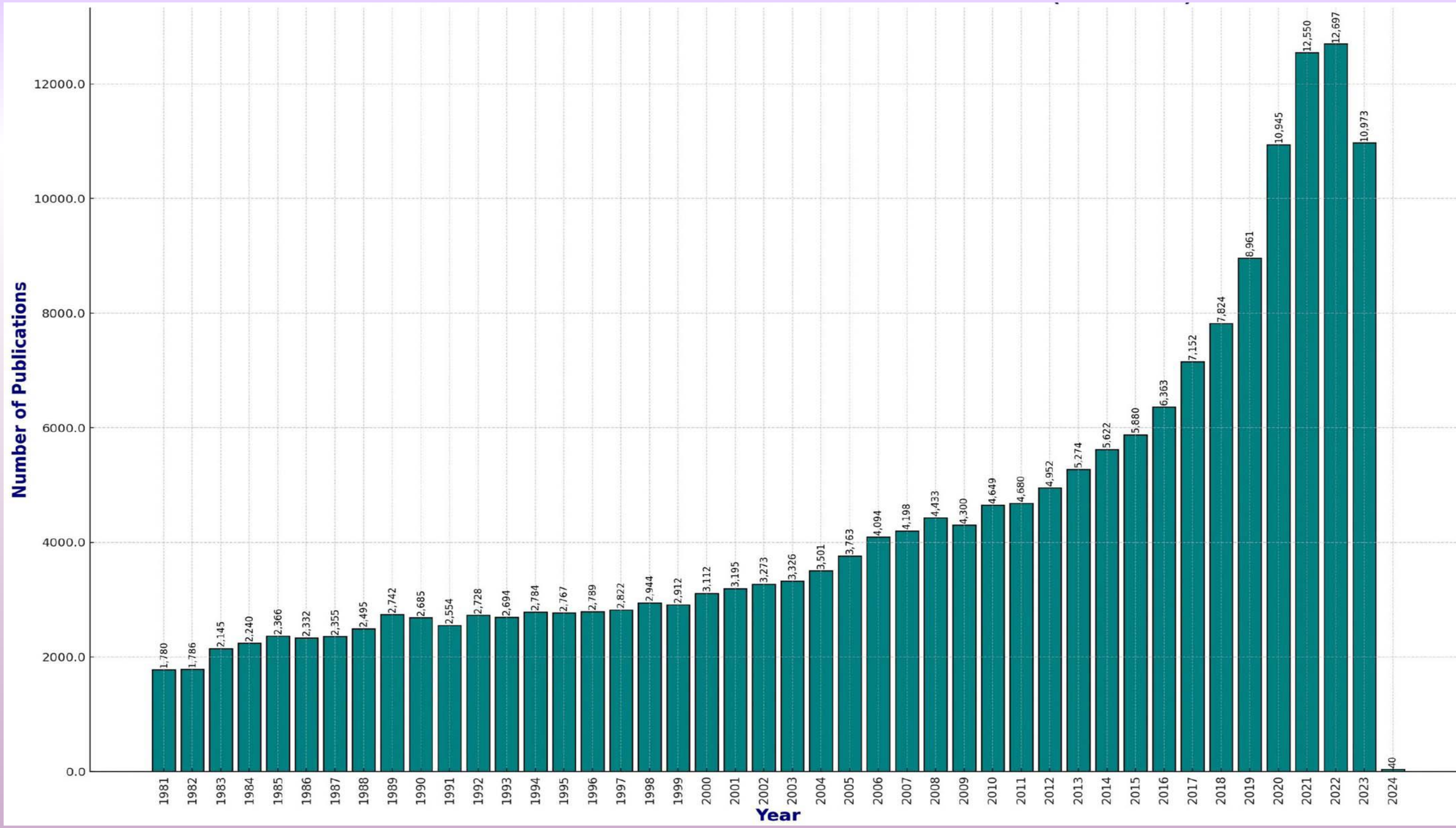


Induce bystander/ abscopal effects including:

- Inflammation
- DNA damage
- Epigenetic changes
- Telomeric erosion
- Metabolic reprogramming
- **EV-mediated transfer of mitochondrial content which alters metabolic and inflammatory responses of recipient cells.**
- Exosomes derived from irradiated cells mediate invasiveness/metastases

Exosomes: a fast-growing field

Publications Trend for Vesicles / Exosomes, 1981–2024; https://pubmed.ncbi.nlm.nih.gov/.



Exponential Growth in the scientific output of EV especially in cancer-relevant studies highlighted the exosomes' implication in both physiological and pathological processes.

Exosomes: a fast-growing field continue..

HOWEVER

Far fewer studies related to the mechanism of exosomes in targeted and non-targeted effects (NTE) of ionizing radiation.

In 2012 we published the 1st evidence of the role of exosomes in NTE both *in vivo* and *in vitro*

***In vitro* publications:**

RADIATION RESEARCH 2012 , 177, 539–545
Possible Role of Exosomes Containing RNA in Mediating Nontargeted Effect of Ionizing Radiation,
Ammar H. J. Al-Mayah, Sarah L. Irons, Ryan C. Pink, David R. F. Carter and Munira A. Kadhim.
Mutation Research 2015 , 772, 38–45
The non-targeted effects of radiation are perpetuated by exosomes
Ammar Al-Mayah,Scott Brighta, Kim Chapmana, Sarah Ironsb, Ping Luoc, David Carter, Edwin Goodwine, Munira Kadhima.
RADIATION RESEARCH 2017, 187, 98–106
Exosome-Mediated Telomeric Instability in Human Breast Epithelial Cancer Cells Post X-Irradiation
Ammar H J Al-Mayah Scott J Bright Debbie A Bowler , Predrag Slijepcevic, Edwin Goodwin and Munira A Kadhim
NASA – THREE , 2018 : https://three.jsc.nasa.gov/articles
The Emerging Role of Exosomes in the Biological Processes Initiated by Ionizing Radiation
Munira A Kadhim, , Scott J Bright, Ammar H J Al-Mayah, and Edwin Goodwin .
***Int. J. Mol. Sci.* 2021, *22*, 11570, Ionising Radiation Promotes Invasive Potential of Breast Cancer Cells: The Role of Exosomes in the Process.** Raheem AL-Abedi**,**

***In vivo* publications :**

Central European Journal of Occupational and Environmental Medicine 2016; 22 (3-4); Biodistribution Investigations of Technetium-Labelled Murine Bone Marrow-Derived Extracellular Vesicles by Nanospect/Ct
Balogh, Polyák, Zsanett , Benedek, Pöstényi, Nagy, Balogh, Sáfrány, Kadhim, Lumniczky ,
Front. Immunol., March 2017 Extracellular Vesicles Mediate Radiation-Induced Systemic Bystander Signals in the Bone Marrow and Spleen. Tünde Szatmári, Bright, Bowler, Kadhim, Sáfrány Lumniczky .
Out-of-field effects: lessons learned from partial body exposure
S. Pazzaglia, et al, M., Radiation and Environmental Biophysics, https://doi.org/10.1007/s00411-022-00988-0

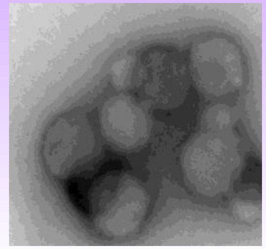
Role of Microvesicales / exsosomes in intercellular communication in NTE

We have tested the hypothesis that microvesicles/ exosomes mediate NTE and that RNA & Proteins play a role in this process.

The Exosome *in vitro* Study Design:

Irradiated Population

Direct irradiated cells



Exosomes collected from Irradiated Condition Media (ICCM)

Exosome- Bystander Population

Non-irradiated bystander cells
(exosome bystander cells)

Initial response

After 20 population doublings

Progeny of irradiated cells

After 20 population doublings

Progeny of exosome bystander cells

Delayed response

Exosome from progeny of irradiated cells

Fresh cells

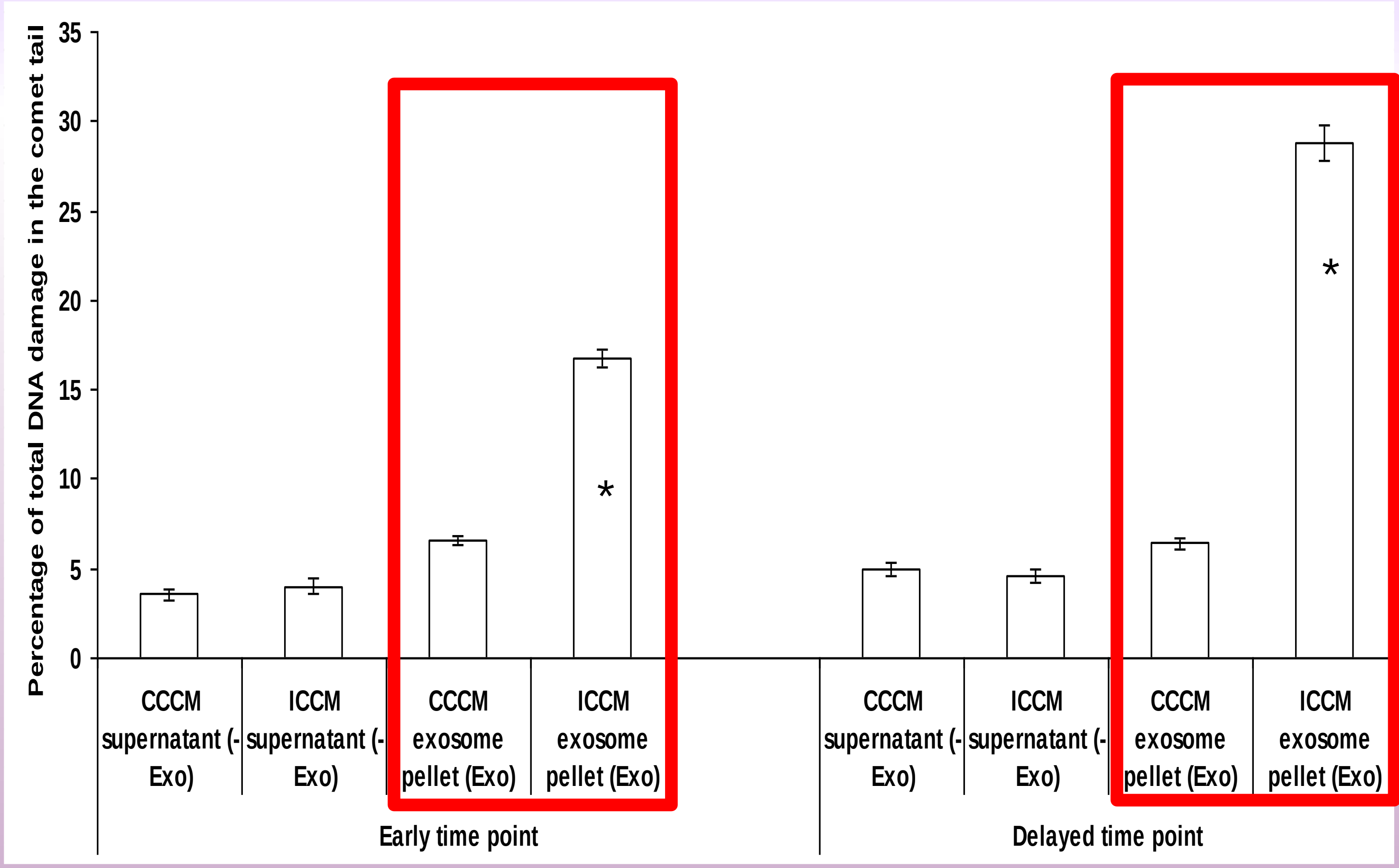
Exosomes collected from the progeny of bystander cells

Fresh cells

Relevant biological end points analysis including DNA damage, Chromosomal and Telomere instability

Al-Mayah et al., 2012, 2015, 2017

Exosomes induced DNA damage in MCF7 cells



The effect is propagated through cell generations and persists in the progeny of both irradiated and bystander populations

Al-Mayah et al. 2012

Characterisation of exosomes and *in vitro* functional effects:

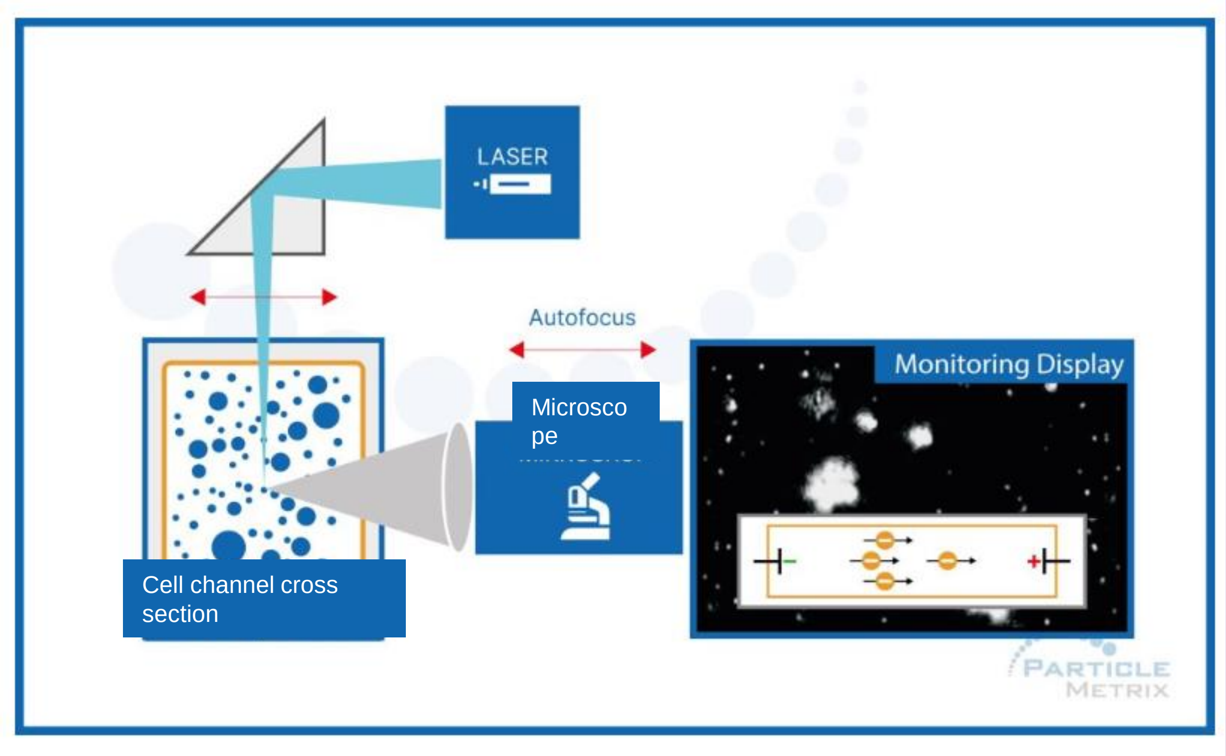
Charecterisation : (q-Nano system)

- Size
- Concentration
- Surface charge
- Small scale RNA analysis

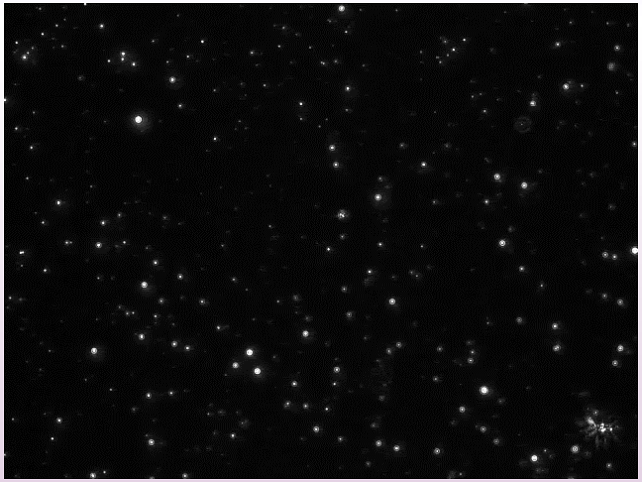
Functional studies

- Imaging
- Intracellular effects
  - DNA damage/apoptosis
  - Immune activation
  - Cell cycle
  - Cell motility

EV/exosome Characterization: Concentration & Size



Zetaview – Nanoparticle tracking analysis **(NTA)**



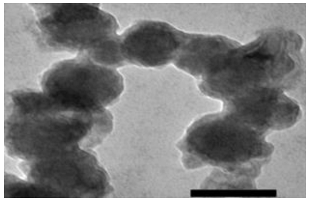
Source:
https://vimeo.com/226831646

- Particles visualized by laser beam.
- Scattered light from the particles recorded by light-sensitive CMOS camera - **ultra microscopy**,
- Size of each particle is calculated by Brownian motion analysis of the individual tracks

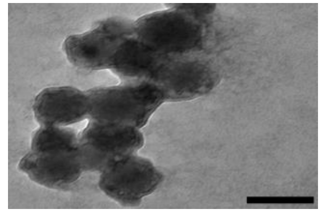
Allows simultaneous determination of size and concentration.

Exosomal characterisation - Electron microscopy & concentration

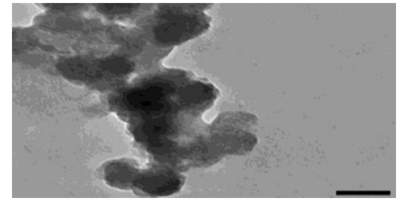
	Size (nm)
	54.8
	51.5
	58.6
	68.9
	60.5
	63.3
	72.9
	74.4
	61.4
	71.7
	55.1
	77.5
	63.4
	75
	45.8
	70.5
	72.1
Average	64.55
Stdev	8.99



Exosomes-Control

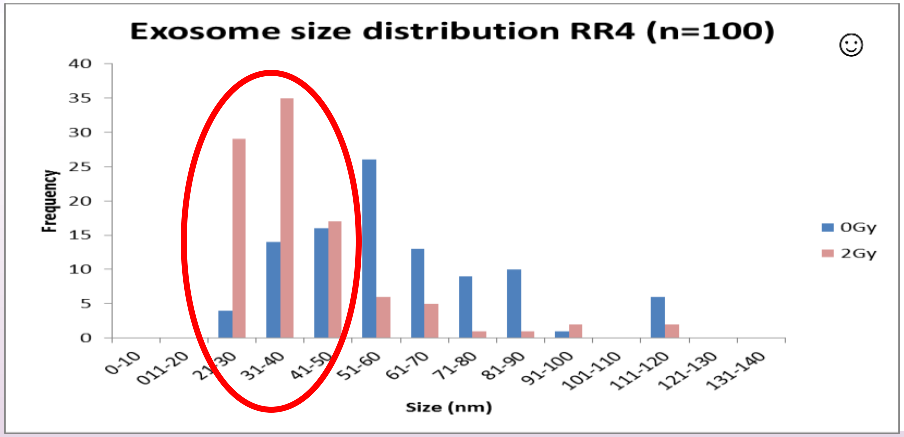


Exosomes- Irradiated cells

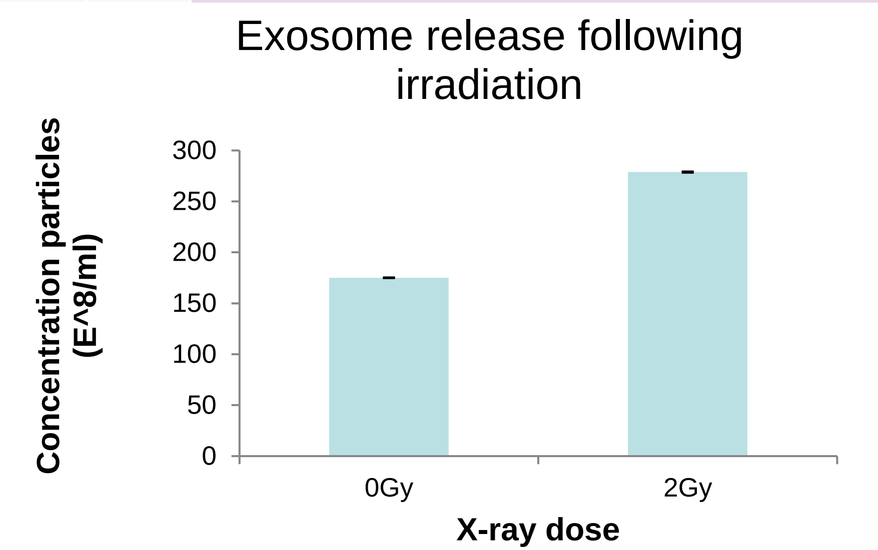


Exosomes- Bystandered cells

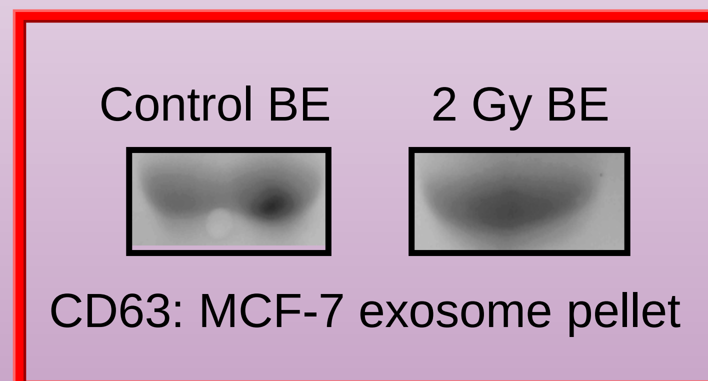
Al-Mayah et al, 2015



☺ Laura Jacobs-PhD project



Western blotting confirmed their endosomal origin



Summary 1:

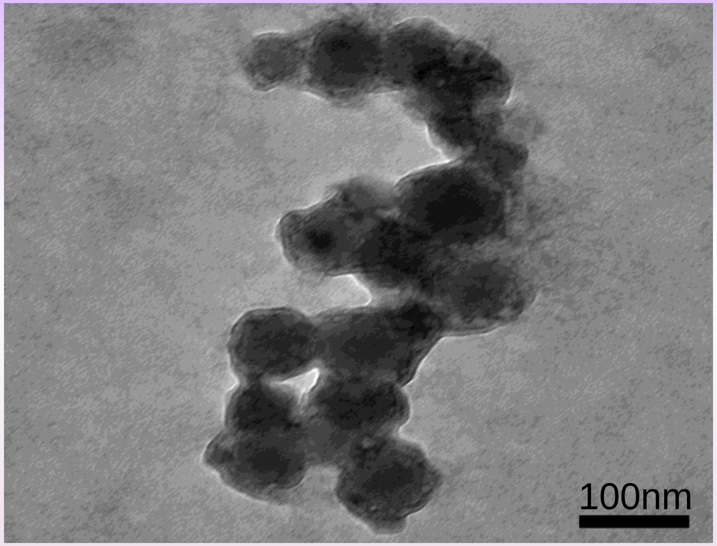
- Exosomes are transmitted factors, involved significantly in the Non Targeted Effects (GI & BE) of radiation exposure.

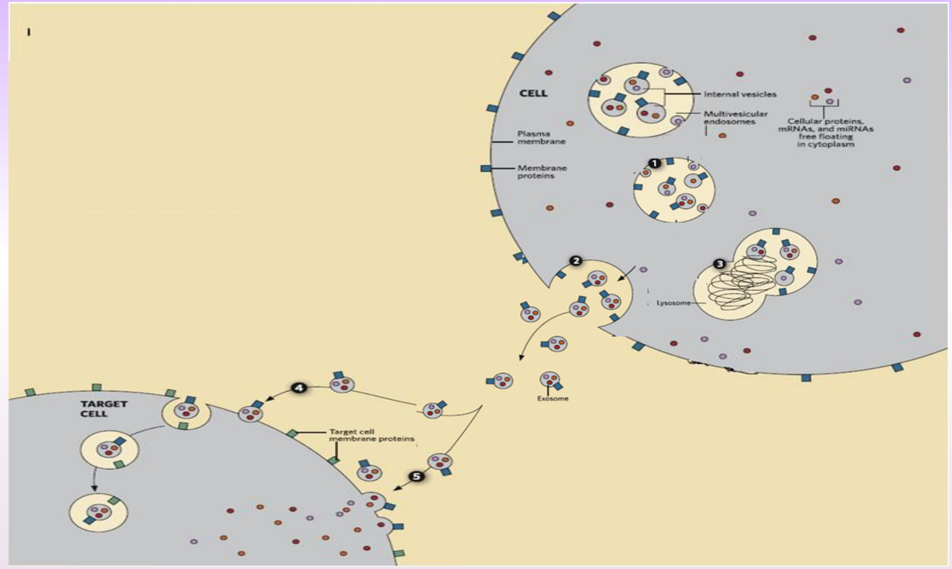
- This effect showed longevity, observed >20 doublings post-irradiation in progeny of irradiated & bystander cells

- Removal of exosomes from irradiated supernatant has shown significant reduction of Chromosomal instability & total DNA damage.

So how this might occur?

EXOSOMES





http://icn.postech.ac.kr/icn_intro_new

- Exosomes are small heterogeneous membrane vesicles (50-150 nm).
- Present in all body fluids (Blood, Urine, Saliva, Milk etc.)
- Cell-cell mediators with physiological & pathological significance
- Specific surface proteins
- **Contain DNA, RNA molecules and protein .**
- Secreted by cells to the extra cellular environment
- Exosomes can be taken up by recipient cells in the delivery of their protein and RNA cargo.
- Cancer cells exosomes can induce oncogenic properties in the recipient cells (increase in cell division or metastatic behaviour) :*Lee et al, 2011, Semin Immunopathol DOI 10.1007/s00281-011-0250-3*

EXOSOME FUNCTIONAL CONTENTS: RNA & Protein Cargo

Hypothesis – Extracellular vesicles could be a vehicle for RIBE/RIGI

In Vitro Studies

Rad.Res. 2012 , 177: 539-545
Possible role of Exosomes Containing RNA in mediating Non-Targeted Effect of Ionizing Radiation.
Al-Mayah, A.H.; Irons, S.L.; Pink, R.C.; Carter, D.R.; Kadhim, M.A.

Mutation Research 2015 , 772, 38–45
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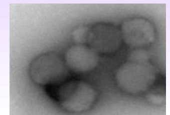
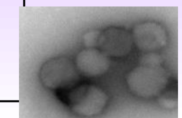
RADIATION RESEARCH 2017, 187, 98–106
Exosome-Mediated Telomeric Instability in Human Breast Epithelial Cancer Cells Post X-Irradiation
Ammar H J Al-Mayah Scott J Bright Debbie A Bowler , Predrag Slijepcevic, Edwin Goodwin Munira A Kadhim

Irradiated cells

Exosome purification





RNA inhibition

Protein inhibition

RNA & protein inhibition

Group 1
Fresh cells

Group 2
Fresh cells

Group 3
Fresh cells

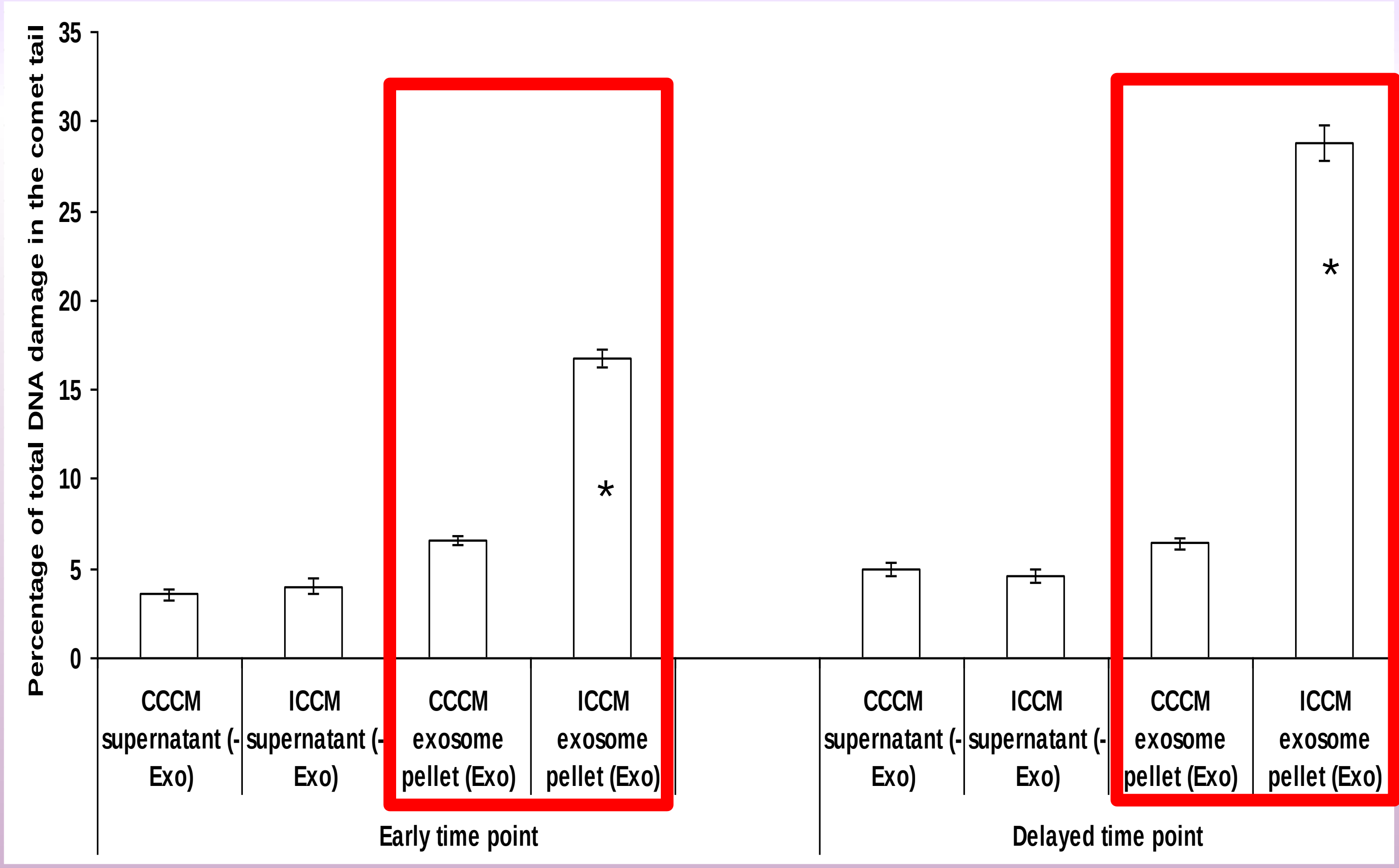
Group 4
Fresh cells

Early biological end point analysis

Cells propagated for delayed response several population doublings later

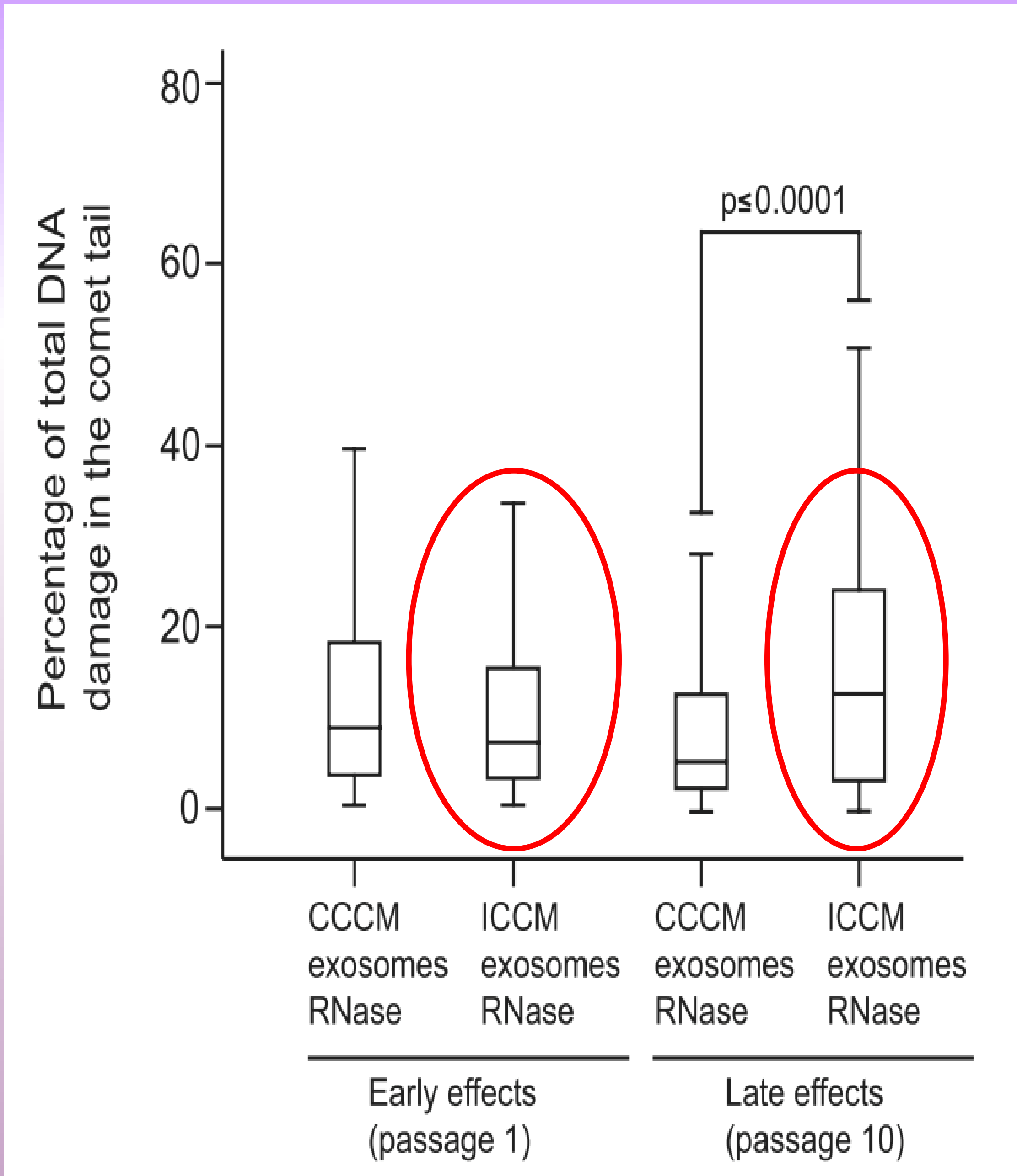
Relevant biological end points analysis including DNA damage, Chromosomal and Telomere instability

Exosomes induced DNA damage in MCF7 cells (Group1)



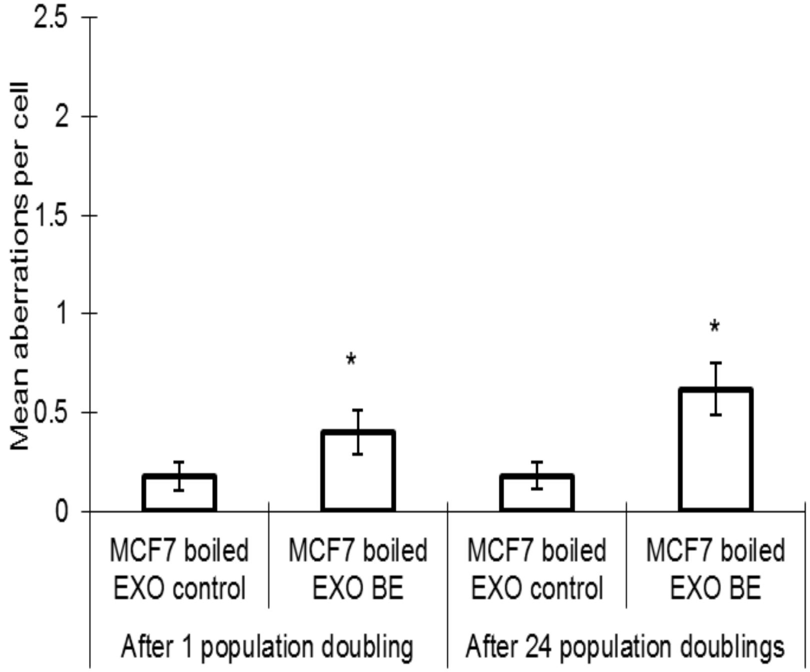
The effect is propagated through cell generations and persists in the progeny bystander populations

Al-Mayah et al. 2012



Group 2

RNAse abolished the effect at the early time point and reduced the effect at the late time point

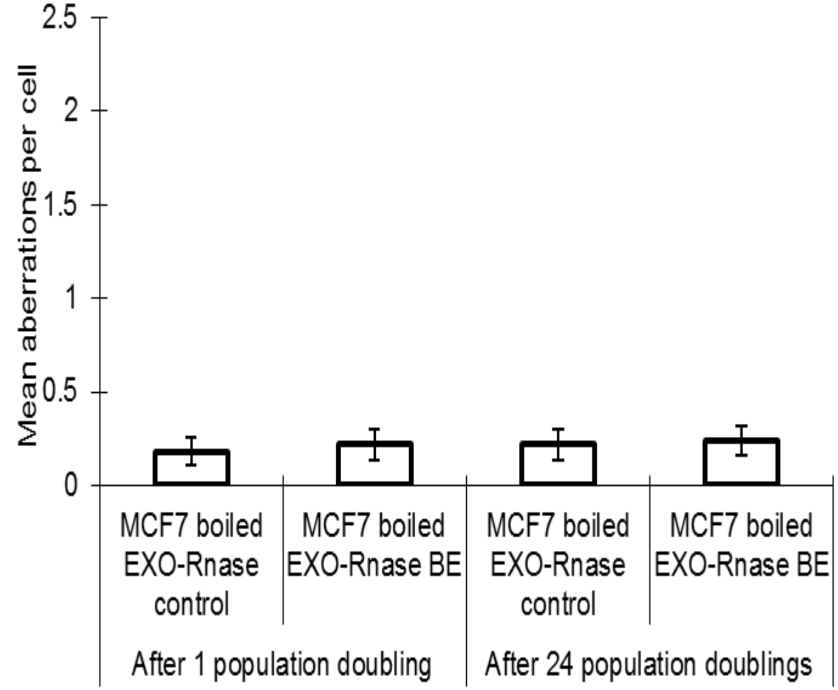


Group 3

Removing protein through boiling wasn't enough to alleviate the effect

Group 4

Removing protein and RNA was enough to alleviate the effect



In vitro: Summary

- Exosomes are significantly involved in the NTE of radiation exposure *in vitro*.
- Both RNA and protein work in a synergistic manner to initiate non-targeted effects of IR.
- Effect is propagated through cell generations and persist in the progeny of both irradiated and bystander populations
- Exosomes are important in this process.

However,

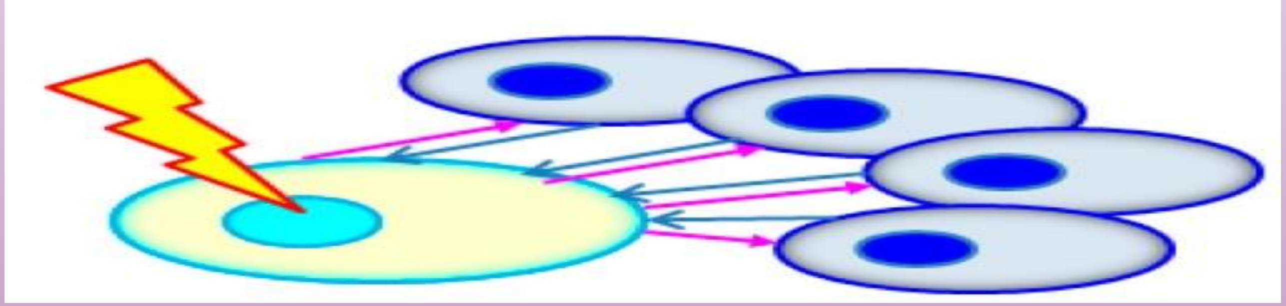
For exosomes/MVs application as biomarkers for risk implication of radiation exposure & radiotherapy , understanding their mechanistic role *in vivo* utmost impotence.

Role of Microvesicles / Exosomes in the induction of NTE : *in vivo* study

In vivo studies

In vivo communication between irradiated and un-irradiated tissues & organs

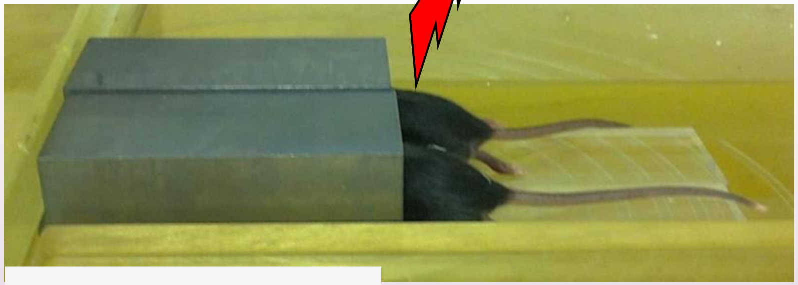
Partial-body irradiation (PBI) exposures are the norm rather than the exception in radiation therapy, imaging , many workplace exposures and **may have significant implications regarding systemic consequences and human health at low and intermediate doses of ionizing radiation**.



Systemic Effects of Partial-body Exposure to Low Radiation Doses

C57BL/6 mice Irradiation at 8-12 weeks of age with X-ray doses of 0, 0.1, and 2 Gy

Partial body irradiation (PBI)



PBI was performed by exposing the lower third of the mouse body, whilst the upper two thirds were shielded with a shield lead.

Whole body irradiation (WBI)

Sham-irradiated (SI)

At 24 hrs and 15 d : Exosomes isolation & characterisation from:

Blood Plasma

Analysis for:

Neuroinflammation, Metabolomics/Raman spectral analysis, Proteomics & Exosomes functional assays.

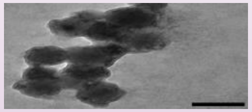
Seda Tuncay Cagatay,et al, Int. J. Mol. Sci. 2020

Exosome characterisation and *in vitro* functional effects

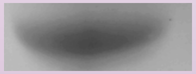
Seda Tuncay Cagatay, et al, Int. J. Mol. Sci. 2020, 21, 8389 ; Simonetta Pazzaglia, et al, Int. J. Mol. Sci. 2022, 23, 2169

Characterisation (q-Nano & NTA system).

- Size and concentration
- Imaging (EM)



- Surface charge CD63



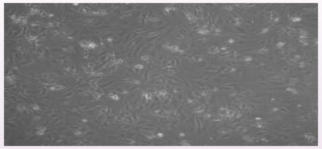
- Small-scale RNA analysis

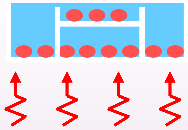
Exosome characterisation and data analysis/NTA

https://vimeo.com/226831646

Functional studies

- In vitro co-culture

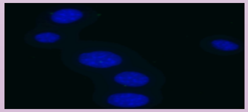




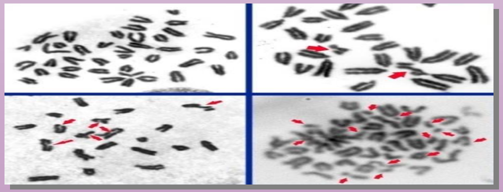
MEF (C57BL/6) [MEFBL/61]

- Cell viability
- DNA damage
  - Comet assay

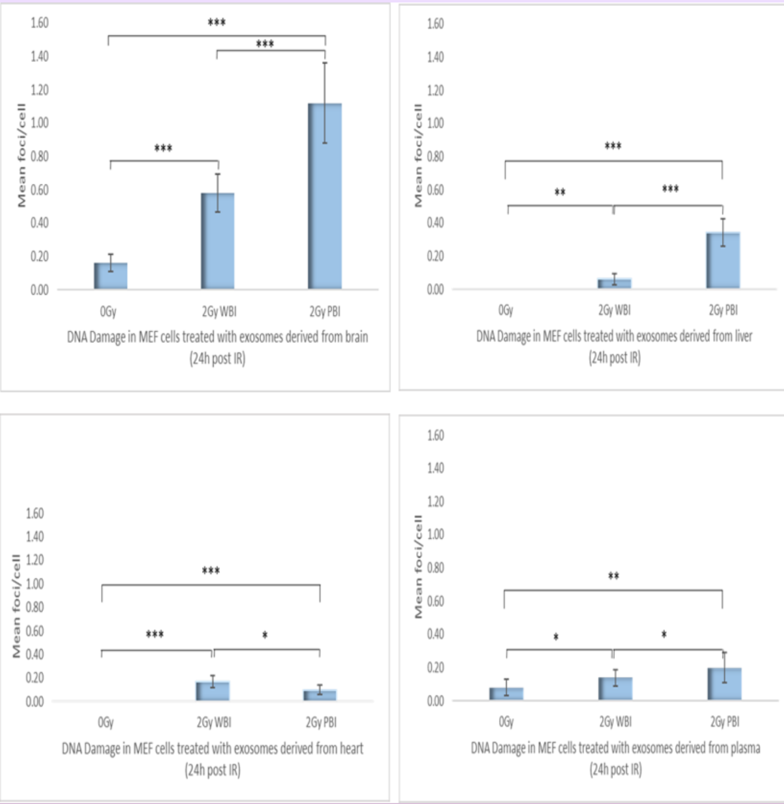
  - γH2AX

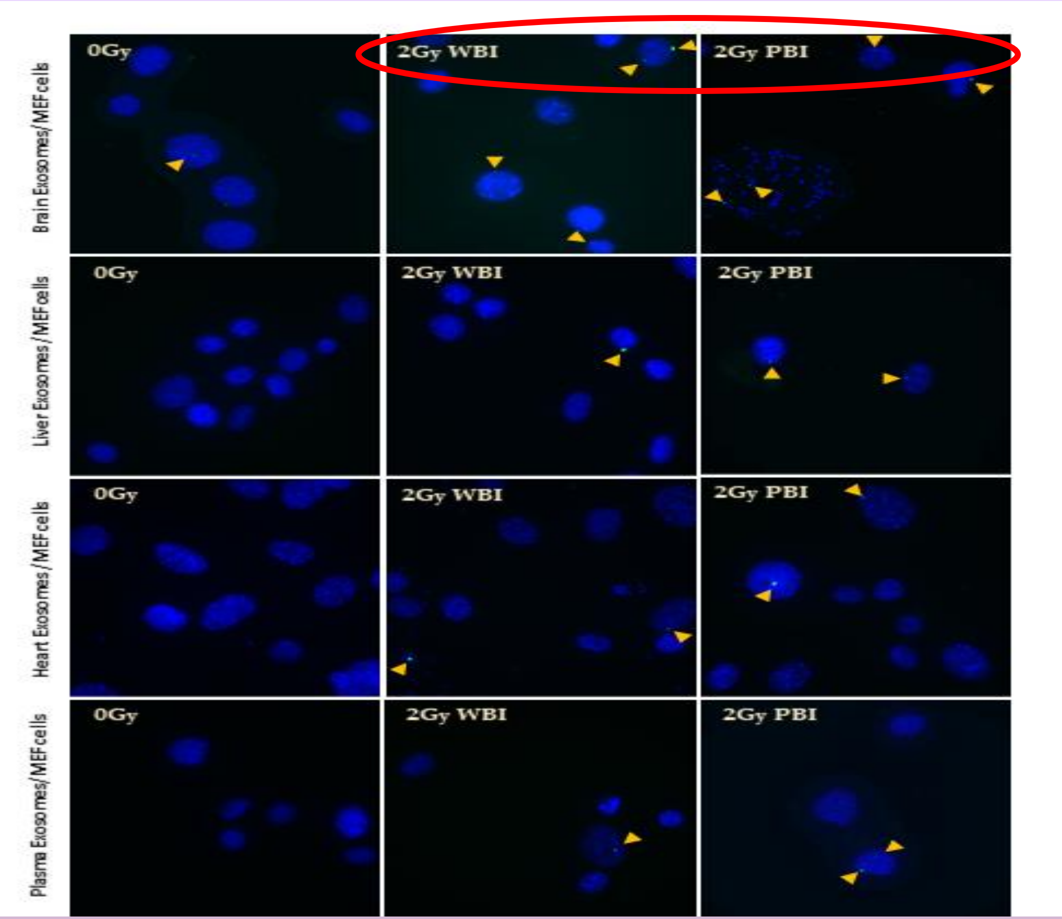


- Chromosomal Instability



The effect of exosomes from organs and plasma of 2Gy WBI & PBI post IR in the induction of DSB in the recipient bystander cells by γH2AX Immunostaining analysis





Highest level of DSBs were observed in MEF cells treated with brain derived exosomes (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Overall Summary

- **Overall,** Ionising radiation can increase the yield of exosomes derived from mouse organs after 24 hours and 15 days under both WBI and PBI irradiation conditions.

- **The exosome functional effects assay** demonstrates the ability of organs' exosomes to induce DNA damage in the treated MEF cells, as shown by γH2AX immunostaining, the comet assay**,** and chromosomal aberrations.

- Specifically, the highest γH2AX foci levels are observed in MEF cells treated with both WBI and PBI **brain exosomes**

- **Exosomes from the plasma** of irradiated mice, prevents radiation-induced apoptosis holding promise for exosome-based future therapeutic applications against radiation injury.

- *In vitro* and *in vivo* studies have shown that exosomes might play a role in out-of-target radiation effects by carrying molecular signalling mediators of radiation damage and having opposite protective functions, resulting in resistance to radiotherapy.

- However, further basic research and technical development will be required.

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- Katalin Lumniczky : NRIRR Hungary
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- Soile Tapio , HMGU , Neuherberg, Germany

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THANK YOU