RC VI.
Challenges in radiation protection research and their radiobiological bases

Katalin Lumniczky
National Public Health Centre, Budapest

The topic of the refresher course is focused on cellular and molecular mechanisms of non-targeted effects of ionizing radiation and how these effects impact direct irradiation effects and modify an individual’s response to ionizing radiation.

Challenges in radiation protection research and their radiobiological bases

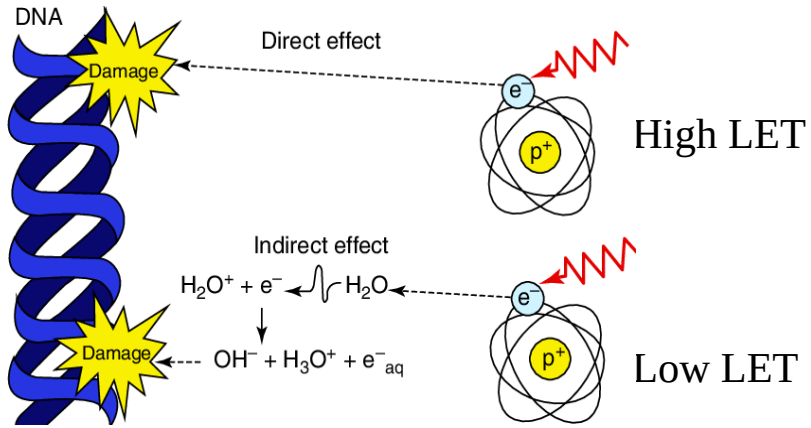
Katalin Lumniczky
National Public Health Centre
Department of Radiobiology and Radiohygiene

The biological effects of ionizing radiation

Targeted effects:

(Cellular damage due to direct absorbsion of ionizing radiation energy within the cells)

DNA targeted effects



Morgan, Sowa, 2005, PNAS

Increased cell death

Increased mutation frequency

Non-DNA targeted effects

Tissue reactions (deterministic effects)

Stochastic (probabilistic) effects (cancer, hereditary effects)

Transcriptional changes

Translational changes

Post-translational changes

Epigenetic changes

Biological assays to detect radiation damage

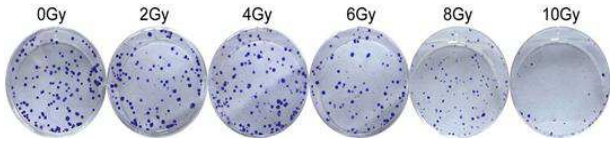
Cell death

Mitotic cell death

- Cells loose their clonogenic potential – failed mitosis
- Slow process
- Cells temporarily maintain some metabolic activity
- Most frequent after IR

Quantification:

Colony forming assay



Liang et al 2018, doi.org/10.2147/CMAR.S176536

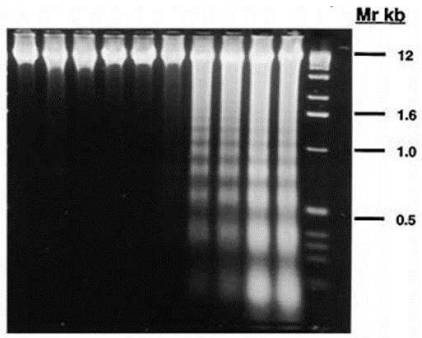
Apoptosis

- Programmed cell death
- Quick process
- Characteristic for certain cell types after IR

Quantification:

Apoptosis detection assays

(DNA fragmentation assays, Annexin, Caspase activation, etc.)



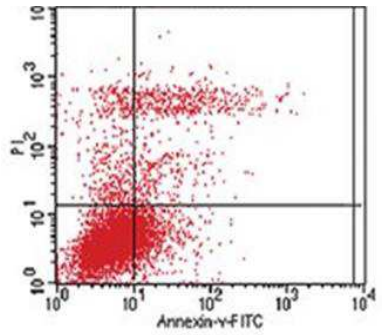
Shao et al 1998, DOI: 10.1074/jbc.272.52.32739

Necrosis

- Cell disintegration
- Mainly after high doses
- Quick process
- Characteristic after high IR doses

Quantification:

Eg. Annexin assay

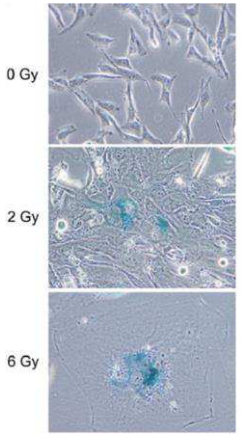


Li et al 2015, DOI: 10.3892/mmr.2015.4466

Senescence

- Permanent cell cycle arrest
- Slow development
- Metabolic and secretory activity maintained
- Characteristic: increased secretory capacity of cells (SASP = senescence-associated secretory phenotype)

Quantification: Eg. β-gal assay



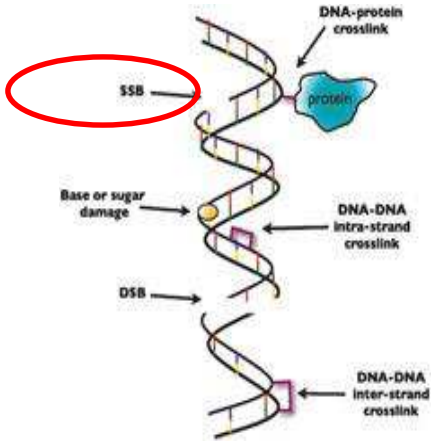
Jinno-Oue et al 2010, DOI: 10.1016/j.ijrobp.2009.08.054

Biological assays to detect/quantify radiation damage

DNA damage

(measuring DNA double-strand breaks and repair kinetics)

Types of DNA damage after IR



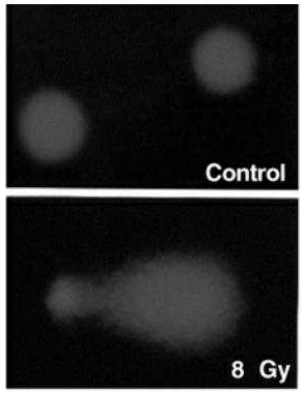
Kavanagh et al 2013, DOI: 10.1089/ars.2012.5151

DNA lesions/cell after 1 Gy X-rays	
Double-strand breaks (DSBs)	40
Single-strand breaks (SSBs)	1000
Base damage	>2000
DNA-DNA crosslinks	30

Direct methods

Pulsed field gel electrophoresis

Comet assay

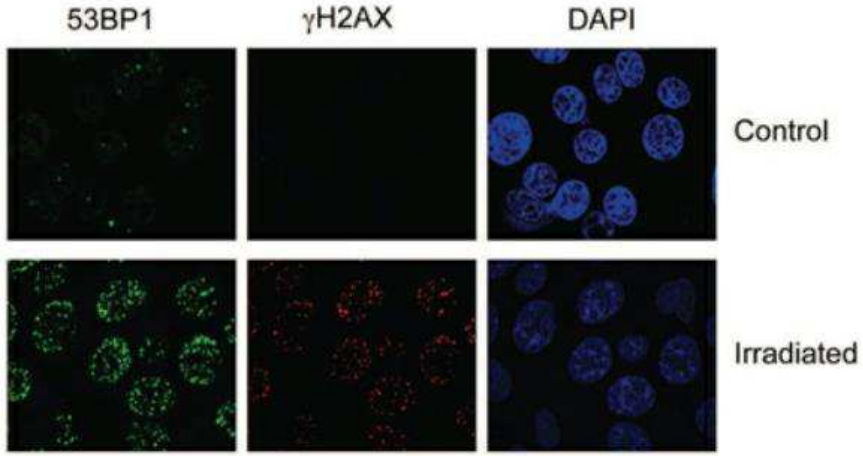


Hall, Garcia, Radiobiology for the radiologist, 8th Edition

Translate into chromosomal aberrations

Indirect methods

Detecting signaling and repair proteins that localize to sites of DNA strand breaks and form foci
(H2AX, 53BP1, ATM, RAD51, BRCA1)

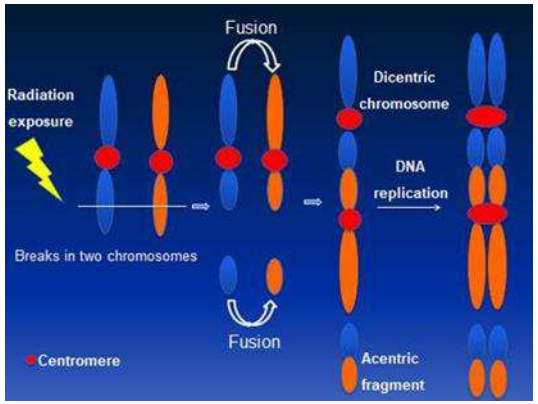


Hall, Garcia, Radiobiology for the radiologist, 8th Edition

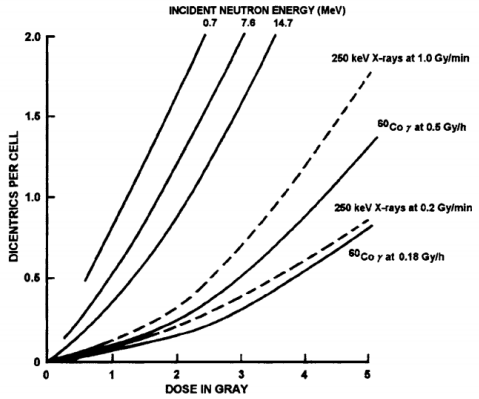
Biological assays to quantify radiation damage

Chromosomal aberrations typical for ionizing radiation exposure

Dicentric chromosomes

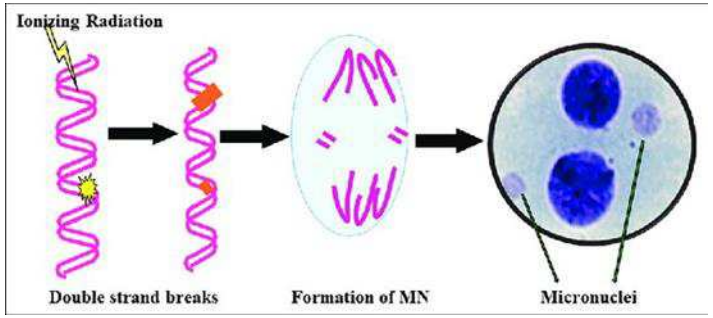


Balajee et al 2018, DOI: 10.21926/obm.genet.1804042

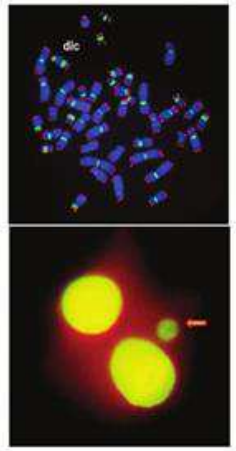


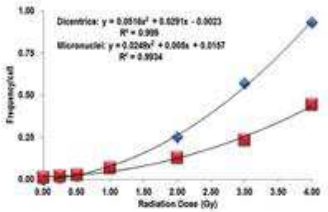
Choppin et al 2002, doi:10.1016/b978-075067463-8/50018-2

Extranuclear DNA fragments (micronuclei)



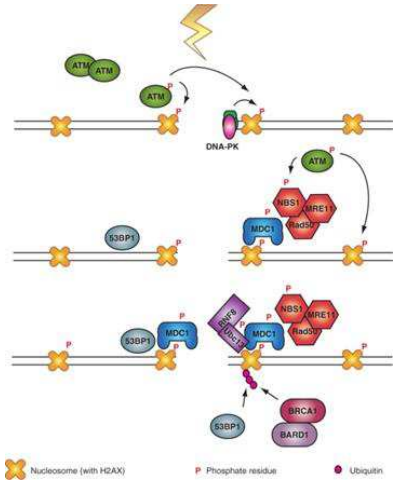
Kanagaraj et al 2017, DOI: 10.4103/jrcr.jrcr_40_17

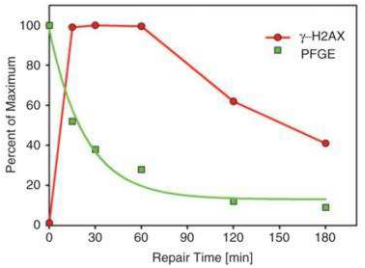




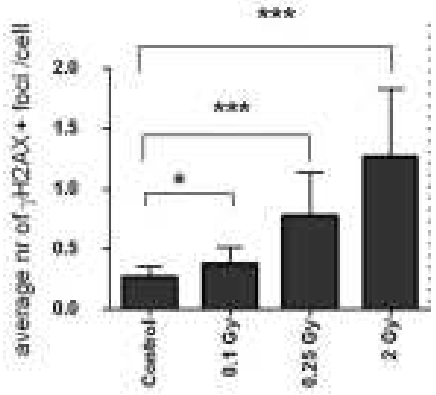
Zeegers et al 2017, DOI: 10.4103/2041-9414.198911

γH2AX foci





Kinner et al 2008, DOI: 10.1093/nar/gkn550



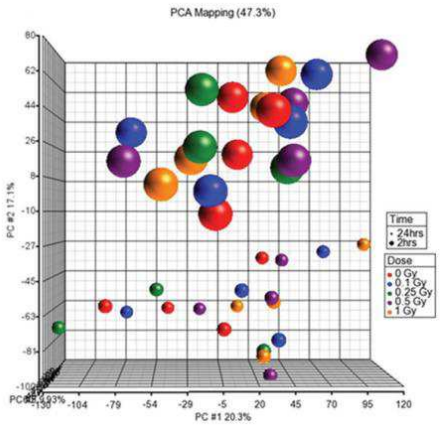
Szatmári et al 2017, DOI: 10.3389/fimmu.2017.00347

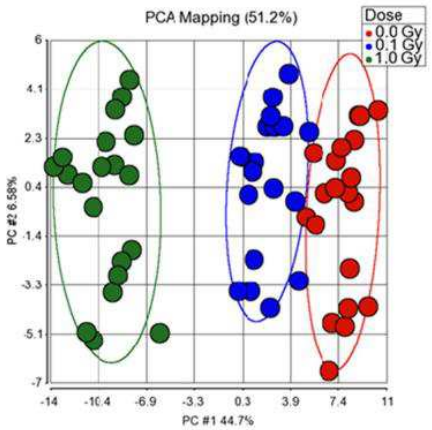
Biological assays to detect/quantify radiation damage

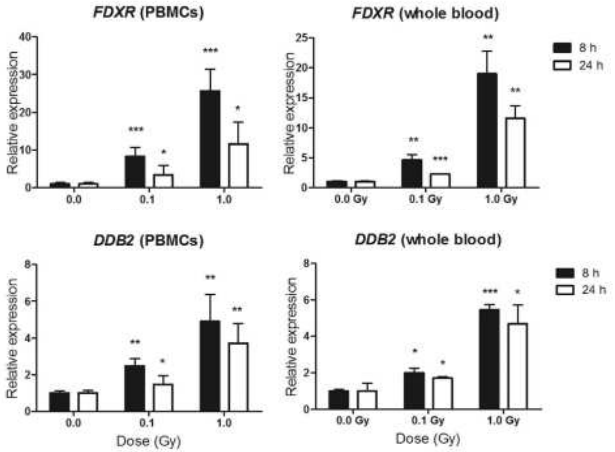
YES ?YES

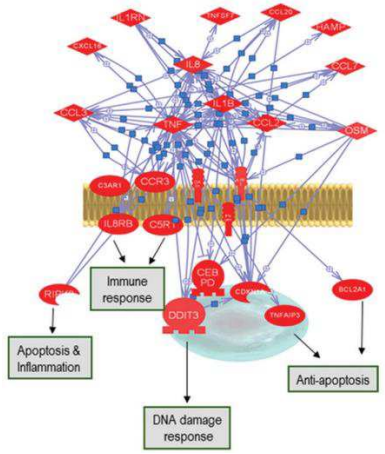
Non-DNA targeted effects

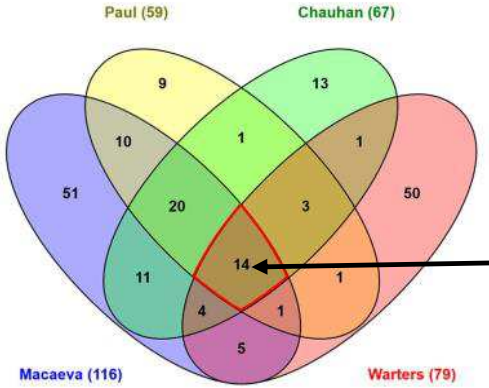
Transcriptional changes
In the blood





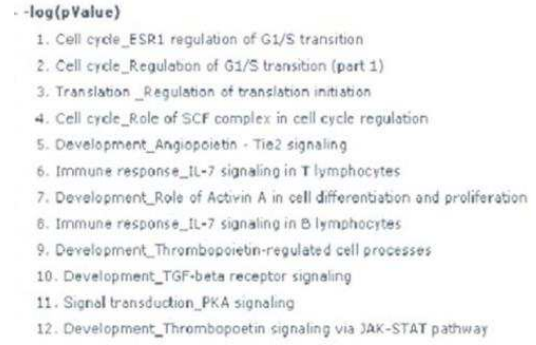


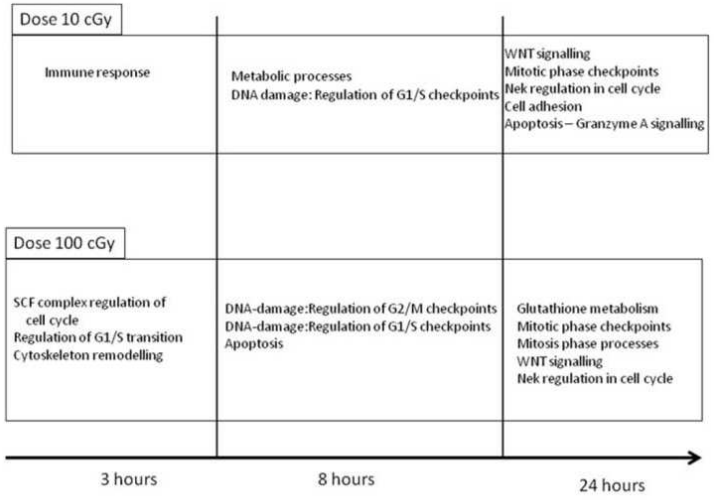




- DDB2*
- POLH
- MDM2*
- RPS27L
- FDXR
- CCNG1*
- TRIAP1
- SESN1*
- FBXO22*
- PPM1D*
- ANKRA2*
- CDKN1A*
- TRIM22*
- BBC3

Transcriptional changes
In the skin





Zeegers et al 2017, DOI: 10.4103/2041-9414.198911

Macaeva et al 2016, DOI: 10.1038/srep19251

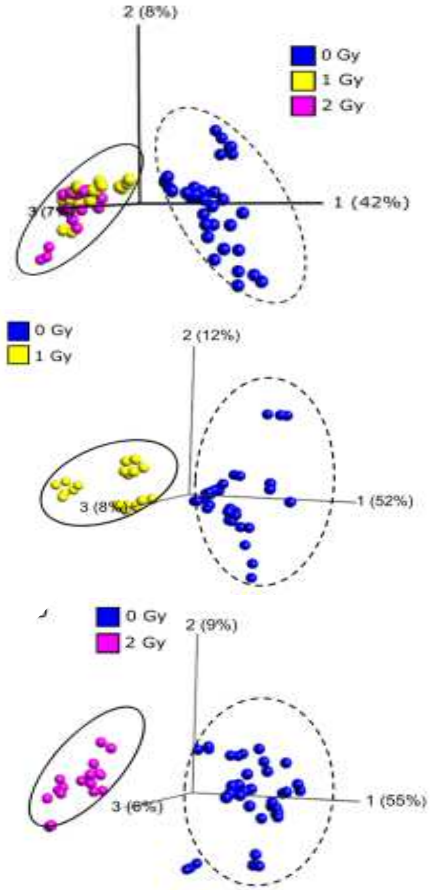
Ray et al 2012, DOI: 10.1186/1471-2164-13-190

Biological assays to detect/quantify radiation damage

?YES ?YES

Non-DNA targeted effects

Changes in the proteome (quantitative protein changes)



Accession no.	Protein name (Gene symbol)	Fold change[a] 1 Gy	2 Gy	adjusted p value[b]
Upregulated proteins				
Q9H2G4	Testis-specific Y-encoded-like protein 2 (TSPYL2)	14.97 ± 5.50	15.26 ± 5.69	3.81E-12[c]
P22570	NADPH:adrenodoxin oxidoreductase, mitochondrial (FDXR)	2.85 ± 0.23	3.47 ± 0.33	6.70E-11[c]
H0YNJ6	GMP reductase (GMPR2)	2.23 ± 0.16	2.59 ± 0.17	1.94E-09[c]
P12814	Alpha-actinin-1 (ACTN1)	2.03 ± 0.13	2.56 ± 0.14	1.01E-09[c]
Q07812	Apoptosis regulator BAX (BAX)	2.32 ± 0.28	2.37 ± 0.17	8.96E-09[c]
Q92466	DNA damage-binding protein 2 (DDB2)	2.04 ± 0.20	2.21 ± 0.18	1.12E-08[c]
Q8WWP7	GTPase IMAP family member 1 (GIMAP1)	1.89 ± 0.23	2.02 ± 0.33	1.39E-08[c]
Q6UXH1	Cysteine-rich with EGF-like domain protein 2 (CRELD2)	1.62 ± 0.10	2.01 ± 0.26	1.73E-08[c]
Q9NRX4	14 kDaphosphohistidine phosphatase (PHPT1)	1.77 ± 0.19	1.93 ± 0.22	2.73E-09[c]
P06127	T-cell surface glycoprotein CD5 (CD5)	1.75 ± 0.12	1.82 ± 0.23	1.43E-08[c]
P04083	Annexin A1 (ANXA1)	1.20 ± 0.06	1.73 ± 0.15	2.44E-08[c]
Q01831	DNA repair protein complementing XP-C cells (XPC)	1.29 ± 0.10	1.58 ± 0.12	2.19E-07[c]
P21291	Cysteine and glycine-rich protein 1 (CSRP1)	1.49 ± 0.10	1.52 ± 0.14	4.73E-07[c]
P17612	cAMP-dependent protein kinase catalytic subunit alpha (PRKACA)	1.28 ± 0.09	1.52 ± 0.02	2.28E-09[c]
A0A087WZM2	Ribonuclease T2 (RNASET2)	1.26 ± 0.05	1.48 ± 0.10	2.98E-09[c]
P08311	Cathepsin G (CTSG)	1.64 ± 0.20	1.44 ± 0.13	2.71E-08[d]
Q9UHD8	Septin-9 (SEPT9)	1.22 ± 0.06	1.36 ± 0.07	4.09E-08[c]
Q96HC4	PDZ and LIM domain protein 5 (PDLIM5)	1.21 ± 0.09	1.34 ± 0.08	1.73E-08[c]
P19367	Hexokinase-1 (HK1)	1.09 ± 0.05	1.34 ± 0.09	2.02E-08[c]
P08133	Annexin A6 (ANXA6)	1.15 ± 0.06	1.30 ± 0.05	4.20E-08[c]
P21283	V-type proton ATPase subunit C 1 (ATP6V1C1)	1.26 ± 0.09	1.29 ± 0.07	1.74E-07[c]
P48426	Phosphatidylinositol 5-phosphate 4-kinase type-2 alpha (PIP4K2A)	1.30 ± 0.06	1.25 ± 0.05	1.04E-07[d]
P20073	Annexin A7 (ANXA7)	1.15 ± 0.06	1.24 ± 0.07	1.12E-07[c]
P46777	60S ribosomal protein L5 (RPL5)	1.09 ± 0.13	1.23 ± 0.18	1.46E-06[c]
O75083	WD repeat-containing protein 1 (WDR1)	1.14 ± 0.05	1.21 ± 0.07	1.98E-08[c]
Q9Y490	Talin-1 (TLN1)	1.20 ± 0.05	1.20 ± 0.06	5.18E-08[c]
A0A024R4M0	40S ribosomal protein S9 (RPS9)	1.30 ± 0.11	1.19 ± 0.11	3.00E-08[d]
A0A0A0MT22	Protein tyrosine phosphatase, receptor type, C, isoform CRA_d (PTPRC)	1.22 ± 0.05	1.10 ± 0.07	1.24E-07[d]

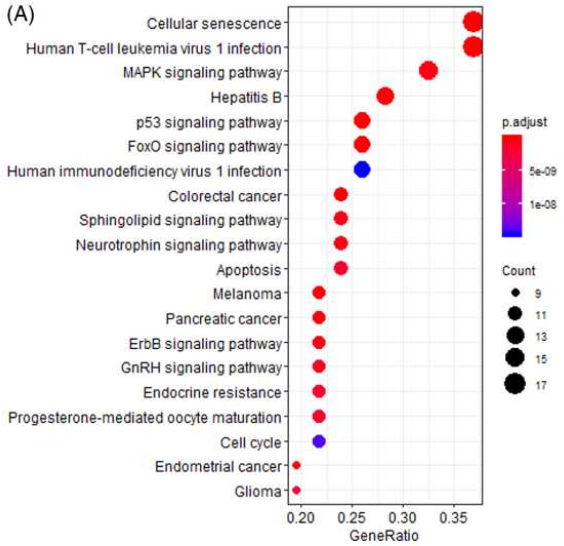
Accession no.	Protein name (Gene symbol)	Fold change[a] 1 Gy	2 Gy	adjusted p value[b]
Down-regulated proteins				
P69905	Hemoglobin subunit alpha (HBA1)	10.91 ± 3.30	12.60 ± 2.94	4.44E-10[c]
P68871	Hemoglobin subunit beta (HBB)	9.64 ± 0.14	10.63 ± 1.31	4.92E-10[c]
O00479	High mobility group nucleosome-binding domain-containing protein 4 (HMGN4)	2.25 ± 0.14	2.16 ± 0.32	8.23E-08[c]
E7EX17	Eukaryotic translation initiation factor 4B (EIF4B)	2.00 ± 0.19	1.89 ± 0.20	1.74E-08[c]
Q9Y2W1	Thyroid hormone receptor-associated protein 3 (THRAP3)	1.92 ± 0.21	1.83 ± 0.14	3.83E-11[c]
P62263	40S ribosomal protein S14 (RPS14)	1.80 ± 0.29	1.82 ± 0.23	8.72E-08[c]
Q9NYF8	Bcl-2-associated transcription factor 1 (BCLAF1)	1.56 ± 0.10	1.82 ± 0.18	7.39E-09[c]
O15400	Syntaxin-7 (STX7)	1.76 ± 0.23	1.65 ± 0.16	3.34E-08[c]
D6RBZ0	Heterogeneous nuclear ribonucleoprotein A/B (HNRNPAB)	1.61 ± 0.12	1.62 ± 0.05	1.94E-11[c]
P38159	RNA-binding motif protein, X chromosome (RBMX)	1.56 ± 0.14	1.57 ± 0.09	6.49E-09[c]
P35611	Alpha-adducin (ADD1)	1.48 ± 0.11	1.46 ± 0.05	9.65E-08[c]
Q96PK6	RNA-binding protein 14 (RBM14)	1.36 ± 0.07	1.43 ± 0.06	4.91E-10[c]
O75400	Pre-mRNA-processing factor 40 homolog A (PRPF40A)	1.37 ± 0.07	1.42 ± 0.07	9.52E-09[c]
Q00839	Heterogeneous nuclear ribonucleoprotein U (HNRNPU)	1.28 ± 0.05	1.40 ± 0.08	4.29E-09[c]
X6R4W8	BUB3-interacting and GLEBS motif-containing protein ZNF207 (ZNF207)	1.25 ± 0.10	1.39 ± 0.13	1.57E-08[c]
Q12874	Splicing factor 3 A subunit 3 (SF3A3)	1.22 ± 0.04	1.38 ± 0.09	1.27E-08[c]
Q13435	Splicing factor 3B subunit 2 (SF3B2)	1.24 ± 0.08	1.32 ± 0.08	3.35E-08[c]
Q9NR30	Nucleolar RNA helicase 2 (DDX21)	1.17 ± 0.03	1.29 ± 0.04	1.01E-08[c]

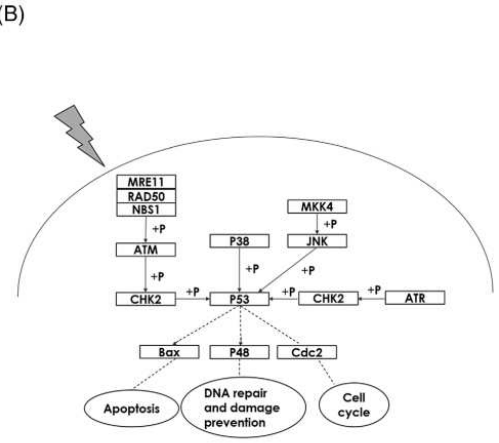
Lee et al 2018, DOI: 10.1038/s41598-018-31740-8

Biological assays to detect/quantify radiation damage

Non-DNA targeted effects

Post-translational modifications – protein phosphorylation



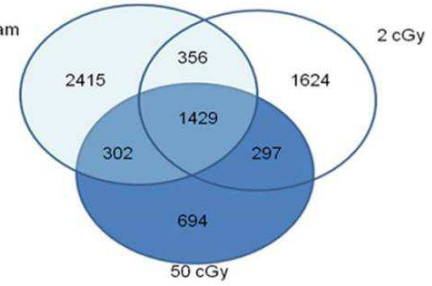


Liu et al 2019, DOI: 10.1002/cbf.3467

Kinases and regulators affected by radiation.

Gene	Peptide	T-test: 2 cGy	T-test: 50 cGy	Change2 cGy	Change 50 cGy
ABL1	K.GQGESDPLDHEPAVS*PLLPR.K	**0.0113**	**0.0008**	2.6	2.9
AKAP11	R.SVS*PTFLNPSDENLK.T	0.1771	**0.0046**	2.7	2.0
MAPK1	R.VADPDHDHTGFLTEY*VATR.W	**0.0009**	**0.0031**	2.8	2.5
PDPK1	R.ANS*FVGTAQYVSPELLTEK.S	**0.0043**	**0.0132**	3.5	3.2
PRKAB2	R.DLSSS*PPGPYGQEMYAFR.S	**0.0009**	**0.0106**	3.6	2.7
		G-Test: 2 cGy	G-Test: 50 cGy	Spectra: 0:2 cGy	Spectra: 0:50 cGy
PRKAR2A	VADAKGDS*ES*EEDEDLEVPVPSR	**16.76**	**11.45**	38:11	38:11
AKAP12	VLSKPPEGVVSEVEMLSS*QER	0.52	**7.35**	0:1	0:7
AKAP2	TNGHS*PSQPR	**6.73**	**4.94**	11: 2	11: 2
MAP3K11	NVFEVGPGDS*PTFPR	**5.13**	0.23	5:0	5:3
MAP4K4	RDS*PLQGSGQQNSQAGQR	0.78	**4.37**	8:5	8:1
PRKCDBP	APEPLGPADQSELGPEQLEAEVGES*S*DEEPVESR	0	**37.86**	0:0	0:35
PRKD1	RLS*NVSLTGVSTIR	1.28	**4.13**	5:2	5:0
EGFR	ELVEPLT*PSGEAPNQALLR	**5.13**	**4.13**	5:0	5:0

Phosphopeptides



. KEGG pathways affected by radiation.

KEGG pathway	2 cGy		50 cGy	
	Count	Proteins	Count	Proteins
Insulin signaling pathway	8	MAPK1, PDPK1, IRS2, EIF4EBP1, PRKAR2A, TSC1, PRKAB2, TSC2	6	PDPK1, IRS2, PRKAR2A, EIF4EBP1, TSC1, PRKAB2
Pathways in cancer	7	EGFR, MAPK1, CCDC6, HDAC1, RALBP1, JUN, ABL1	5	EGFR, HDAC1, PML, LOC652671, ABL1
MAPK signaling pathway	6	EGFR, MAPK1, JUN, RRAS, STMN1, MAP3K11	6	EGFR, MAP4K4, NF1, RRAS, NFATC4, STMN1
mTOR signaling pathway	6	EIF4B, MAPK1, PDPK1, EIF4EBP1, TSC1, TSC2	4	EIF4B, PDPK1, EIF4EBP1, TSC1
Tight junction	6	EPB41L2, RAB3B, TJP1, MAGI1, RRAS, TJAP1	4	EPB41L2, MAGI1, RRAS, TJP2
Adherens junction	6	EGFR, MAPK1, TJP1, BAIAP2, LMO7, VCL	3	EGFR, LMO7, CTNND1
Endocytosis	5	EGFR, DAB2, RABEP1, SH3KBP1, IQSEC1	4	EGFR, USP8, SH3KBP1, IQSEC1
Spliceosome	1	SF3B2	6	SFRS4, SFRS9, SNW1, SFRS1, PRPF38B, SF3B2

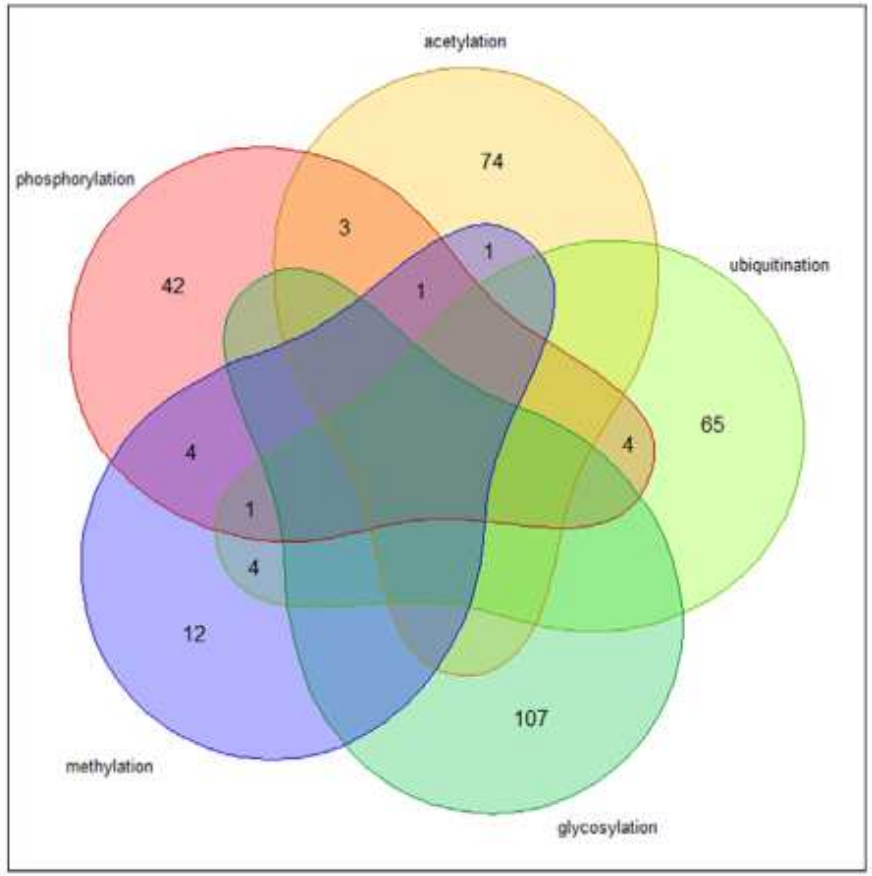
Yang et al 2010, DOI: 10.1371/journal.pone.0014152

Biological assays to detect/quantify radiation damage

YES YES

Non-DNA targeted effects

Post-translational modifications



Number of intersection protein	Symbol	Types of PTMs
1	TP53	phosphorylation ubiquitination methylation
1	ATM	phosphorylation acetylation methylation
1	TP53BP1	acetylation methylation
3	ATR ANTXR1 SERPINA2	phosphorylation acetylation
4	CDKN1A TCEAL1 H3F3AP6 NSG1	ubiquitination methylation
4	BAX CHEK1 MDM2 MRE11	phosphorylation methylation
4	DDB2 H2AFX PARP1 EIF4EBP1	phosphorylation ubiquitination

Liu et al 2019, DOI: 10.1002/cbf.3467

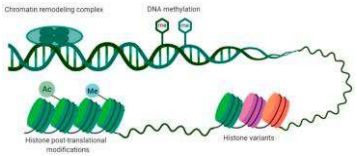
Biological assays to detect/quantify radiation damage

Non-DNA targeted effects

Epigenetic changes miRNA changes

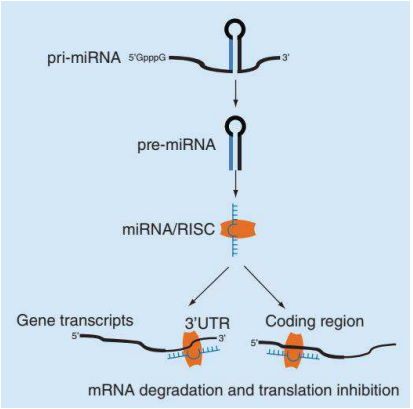
Epigenetics: heritable changes in gene activity, transcript architecture (eg. splice variants), without changes in the DNA sequence

DNA methylation
Histone modifications



Nunes et al 2020, DOI: 10.3390/cells9081850

Small, non-coding RNAs (eg. micro or miRNAs)

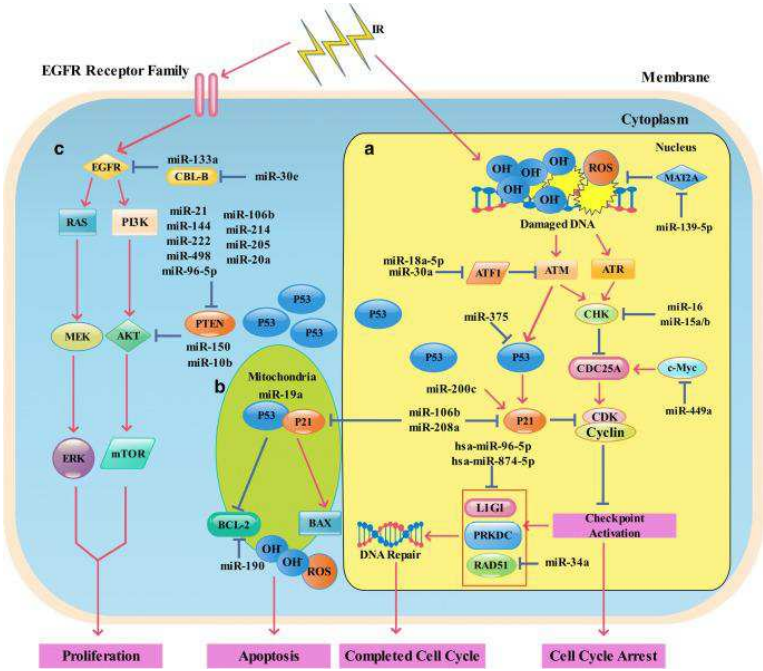


Koturbash et al. 2015, DOI: 10.2217/bmm.15.89

| Serum or plasma miRNAs as IR biomarkers in human samples.

miRNAs	Material	Species	Predictors	References
miR-199a-5p↑	Serum	Human	Radiotherapy prognosis	Baek et al. (2020)
miR-218-5p↑	Serum	Human	–	Chen et al. (2021)
miR-29a-3p↓, miR-150-5p↓	Plasma	Human	IR-induced organ injury	Dinh et al. (2016)
miR-92a-1-5p↓, miR-25-5p↓, miR-1290↑	Serum	Human	Radiotherapy prognosis	Fan et al. (2020)
miR-34a↑	Serum	Human	Dose estimation	Halimi et al. (2016)
miR-26b-5p↓	Serum	Human	Radiotherapy prognosis	Han et al. (2020)
Combination of 16 miRNAs(e.g. miR-100-5p, miR-106b-5p, miR-145-5p)	Serum	Human	IR-induced organ injury	Hawkins et al. (201
miR-143↓	Serum	Human	Radiotherapy prognosis	Hiyoshi et al. (2017)
miR-1281↓, miR-6732-3p↓	Serum	Human	Radiosensitivity	Li et al. (2020a)
miR-6731-5p↑, miR-208b-3p↑, miR-2116-3p↓, miR-574-3p↓etc.	Exosomes in plasma	Human	Radiotherapy prognosis	Li et al. (2020b)
miR-130a↑, miR-25↑, miR-191*↑	Serum	Human, mouse	Radiotherapy prognosis	Lv et al. (2020)
Combination of miR-425-5p, miR-21-5p, miR-106b-5p, miR-590-5p, miR-574-3p, miR-885-3p	Plasma	Human	Dose estimation	Summerer et al. (2013)
miR-142-3p, miR-186-5p, miR-195-5p, miR-374b-5p and miR-574-3p	Plasma	Human	Radiotherapy prognosis	Summerer et al. (2015)
Combination of 11 miRNAs(e.g. miR-10b-5p, miR-125b-5p, miR-126-3p)	Serum	Human	Radiosensitivity	Sun et al. (2018)
miR-208a↑, miR-200a-3p↓, miR-126-3p↓, miR-29b-3p↓	Serum	Human	–	Tang et al. (2016)
Combination of miR-425-5p, miR-185-5p	Serum	Human	IR-induced organ injury	Tomasik et al. (2021
Combination of miR-155, miR-221	Serum	Human	IR-induced organ injury	Xu et al. (2014)
miR-345↓	Serum	Human	Radiotherapy prognosis	Yu et al. (2016)
miR-504↑	Serum	Human	Radiotherapy prognosis	Zhao et al. (2015)

Jia et al. 2022, DOI: 10.3389/fcell.2022.861451



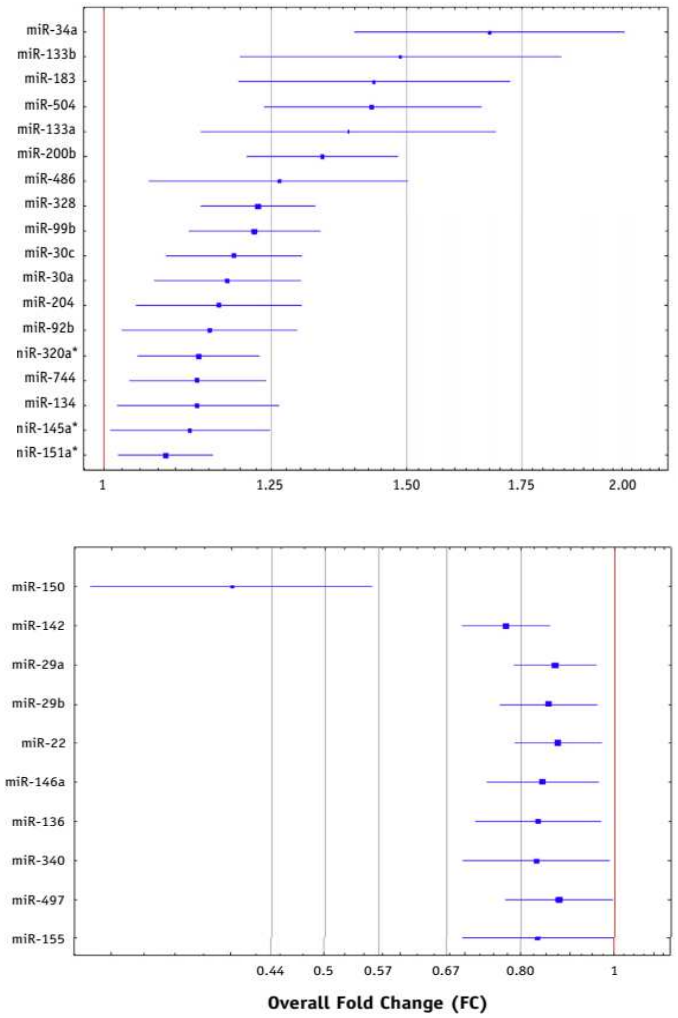
Chen et al. 2021, DOI:10.1007/s11356-021-12509-5

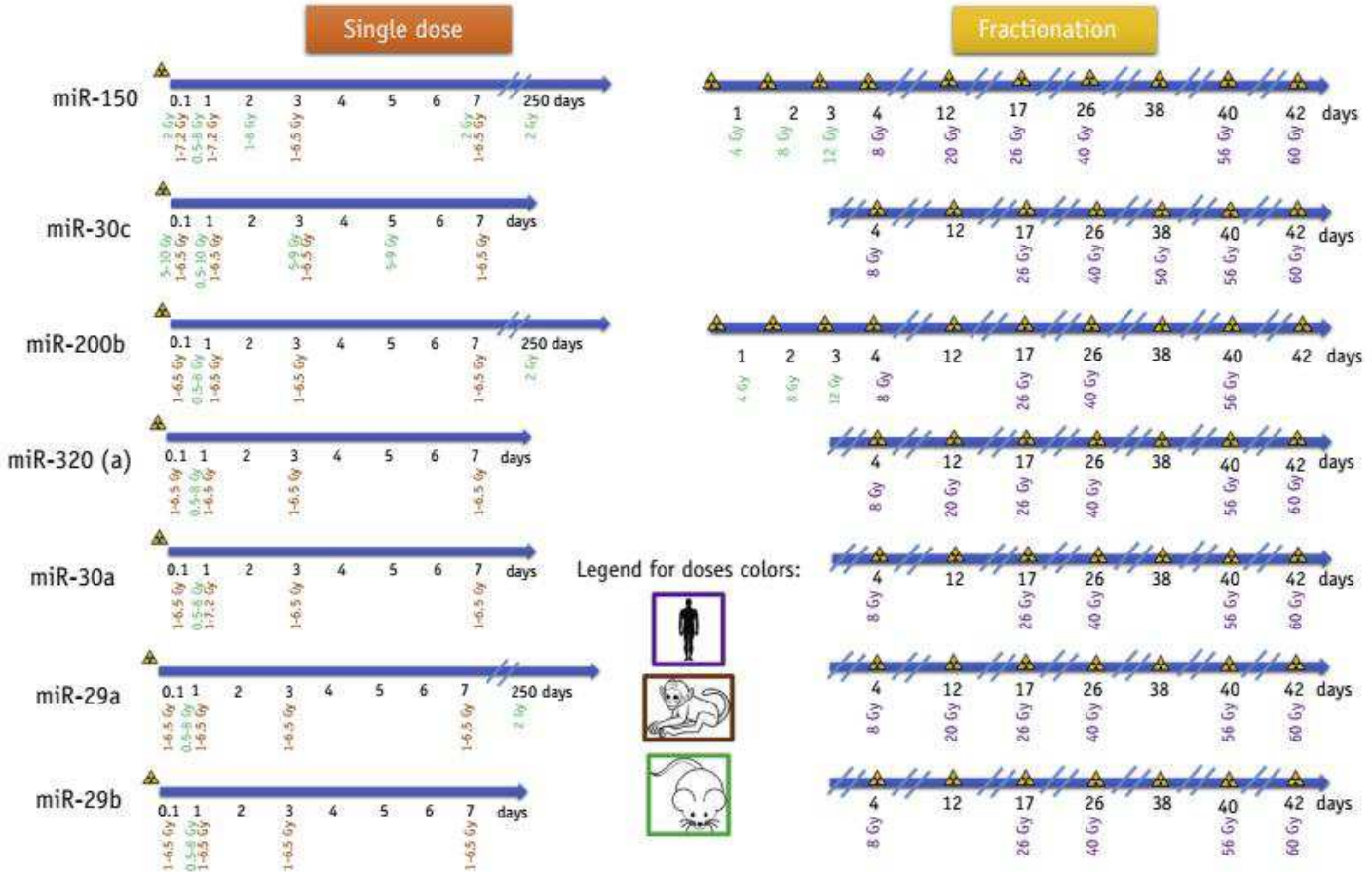
Biological assays to detect/quantify radiation damage

YES ?YES

Non-DNA targeted effects

Epigenetic changes – miRNA changes





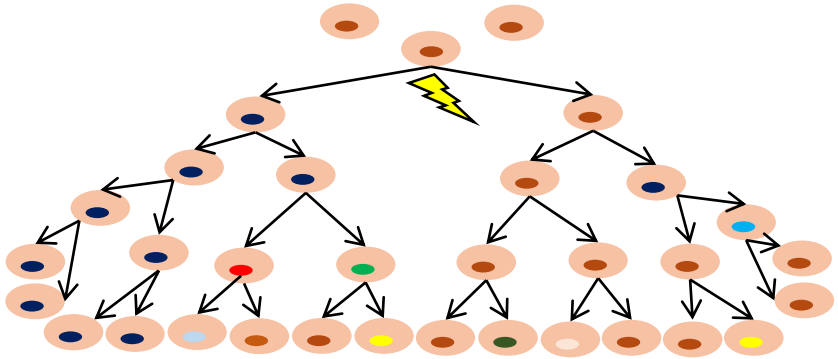
Malachowska et al. 2019, DOI:10.1016/j.ijrobp.2019.10.028

The biological effects of ionizing radiation

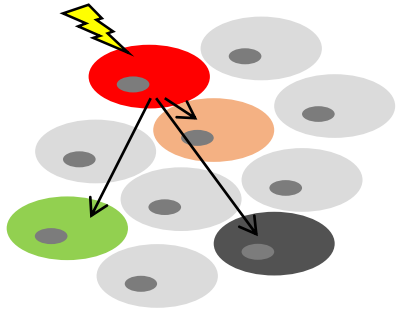
Non-targeted effects:

(Cellular damage in cells not directly hit by ionizing radiation)

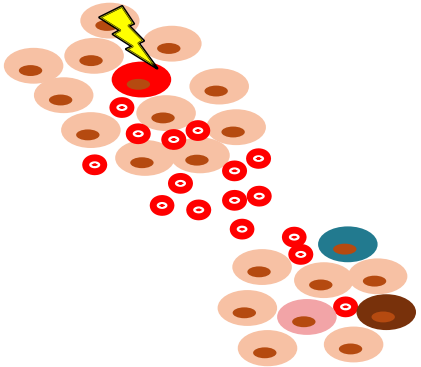
Genetic instability



Bystander effect



Abscopal (systemic or distal bystander) effect



Increased cell death

Increased frequency of chromosomal aberrations

Epigenetic changes

Release of danger signals

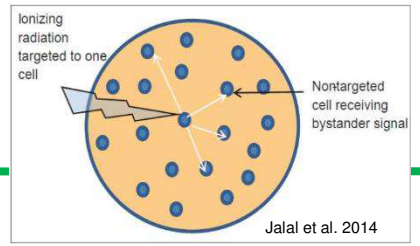
Increased mutation rate

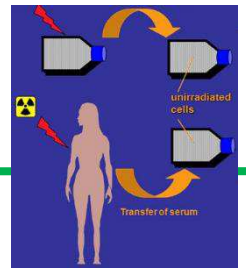
Persistent oxidative stress

Changes in radiation response-related signal transduction pathways

Release of inflammatory and immune modulatory signals

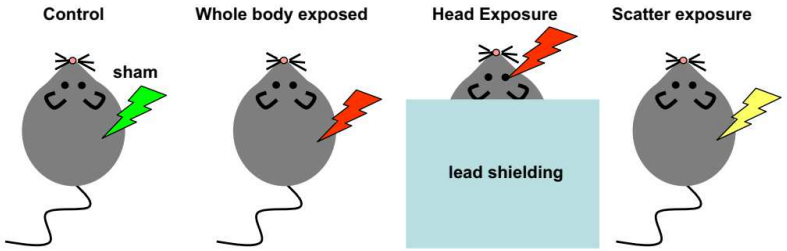
Bystander effects



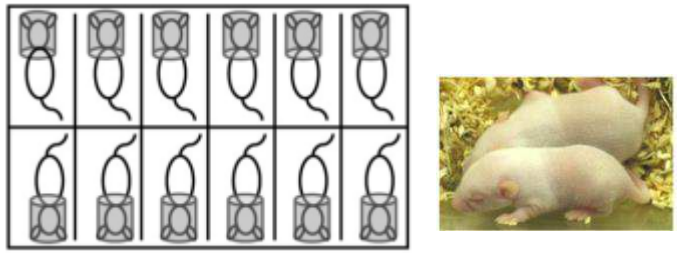


Local head irradiation

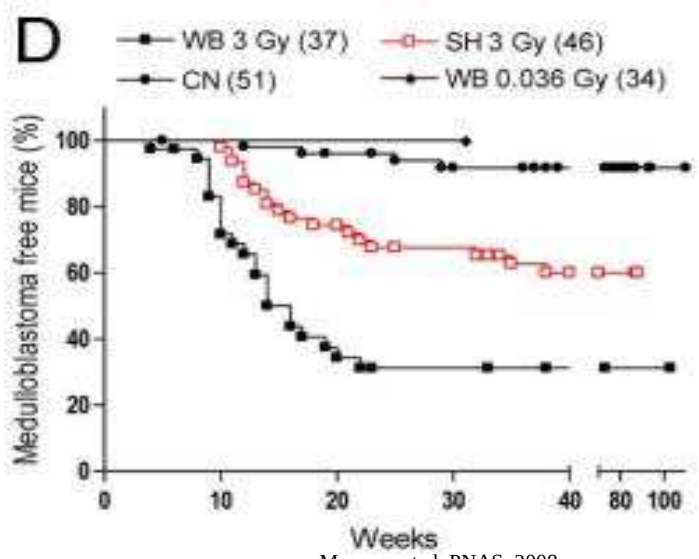
Experimental *in vivo* evidence



Irradiation of the lower part of the body



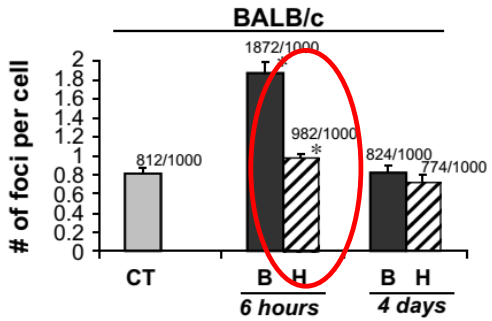
Brain tumors



Mancuso et al. PNAS, 2008

Distant changes in the spleen

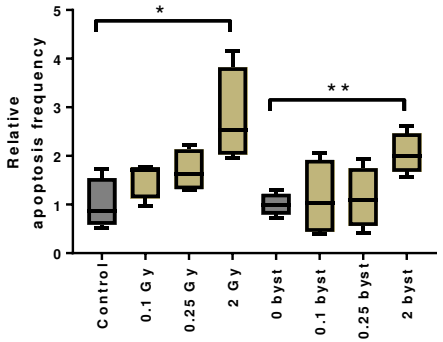
DSBs



Koturbash et al. IJROBP, 2008

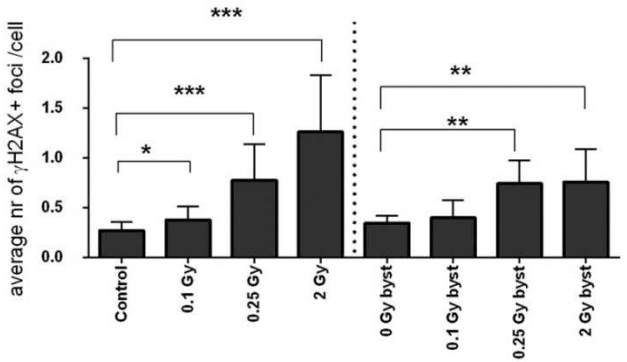
Mice treated systemically with extracellular vesicles originating from irradiated mice

Apoptosis



Kis et al. Cells, 2022

DSBs



Szatmári et al. Front Immunol, 2017

Bystander effects

Hypothetical mechanism

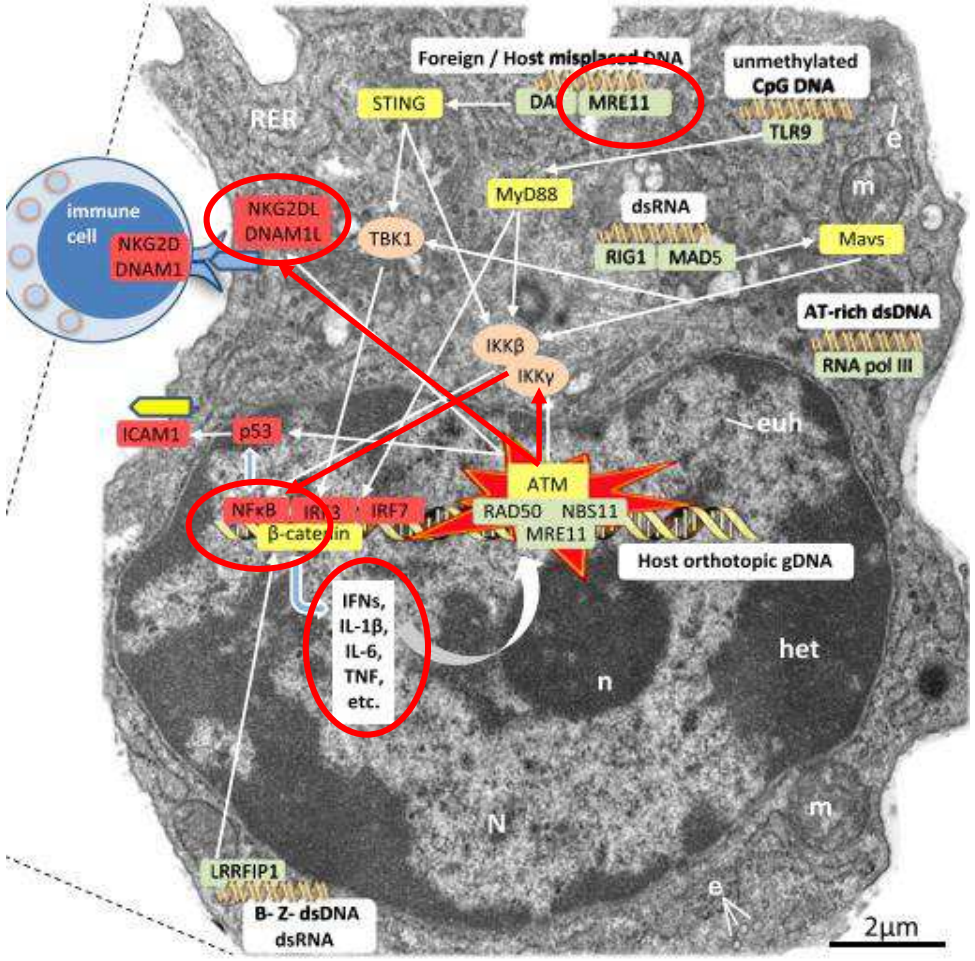
Senescence → SASP

Immunogenic cell death (necrosis, apoptosis)

DNA damage and DDR (DNA damage response)

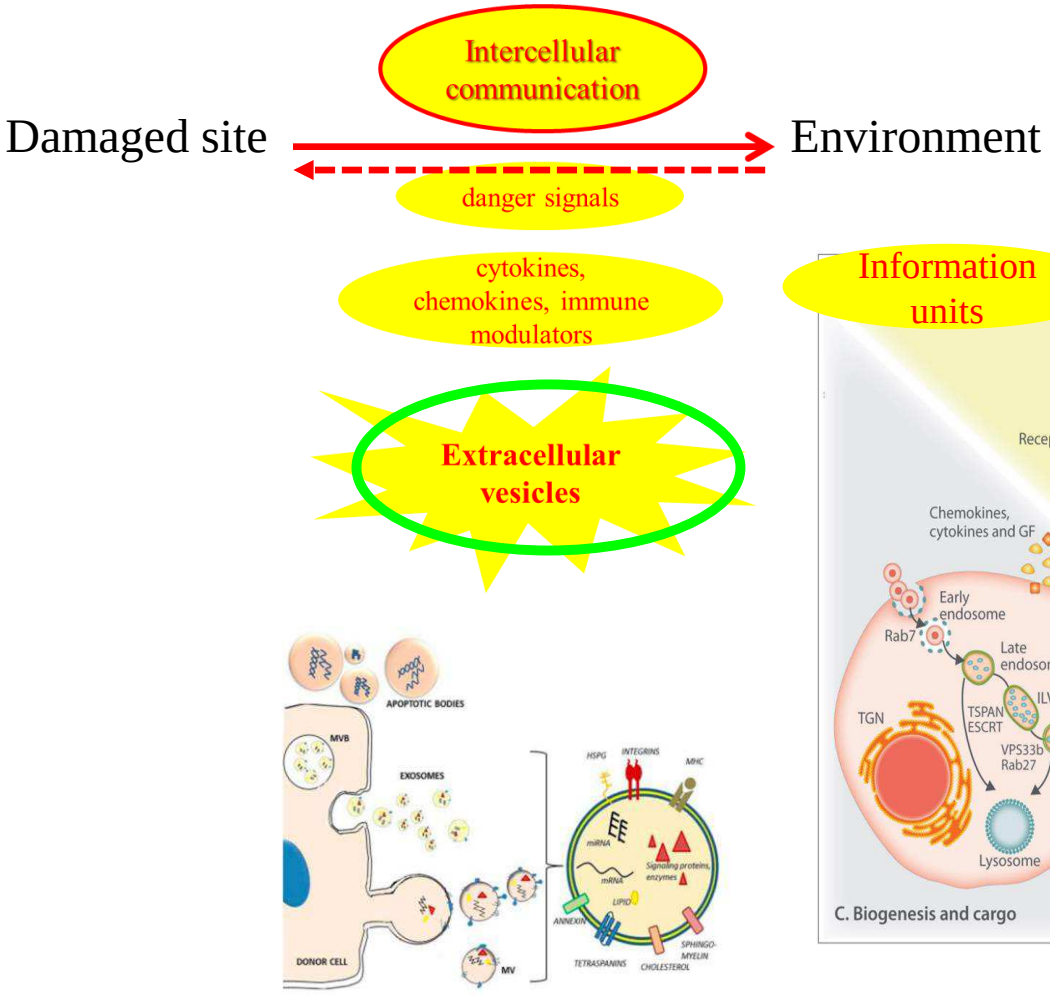
→ Release of danger signals, DAMPs
→ Release of pro-inflammatory factors
→ Calreticulin on cell surface

IRRADIATED CELLS COMMUNICATE WITH THEIR ENVIRONMENT

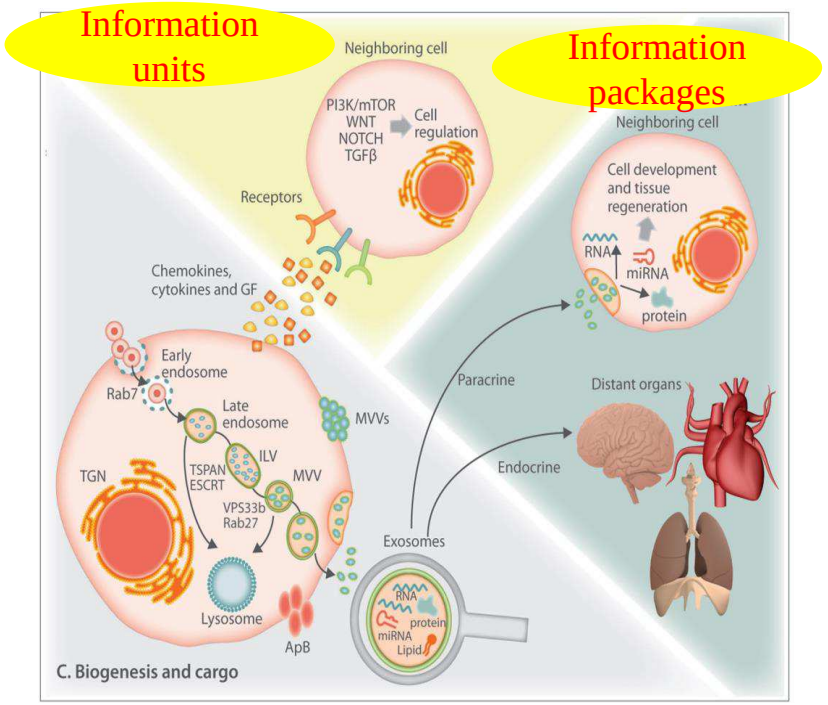


Pateras et al. Pharmacol & Therapeutics 2015

Bystander effects



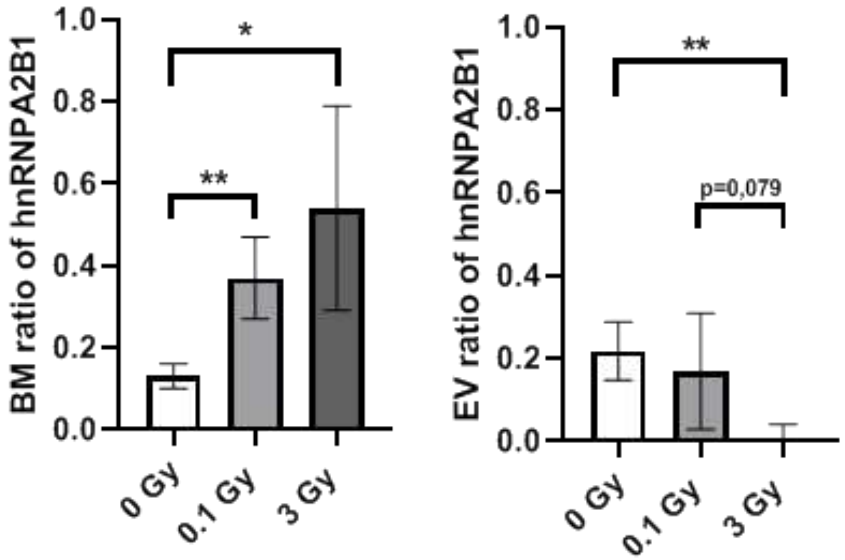
Szatmári et al. IJMS 2020



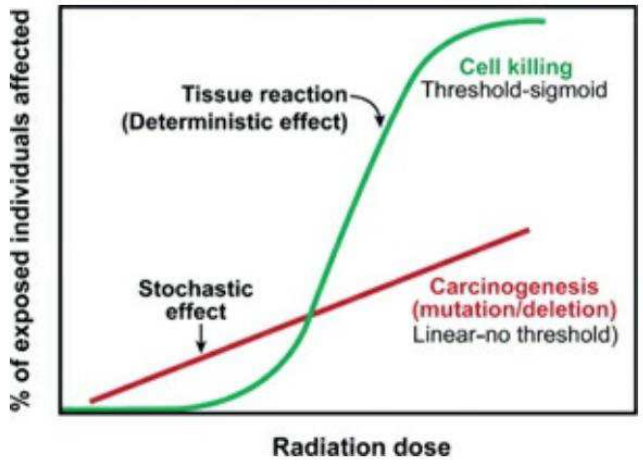
Butler et al, Haematologica, 2018

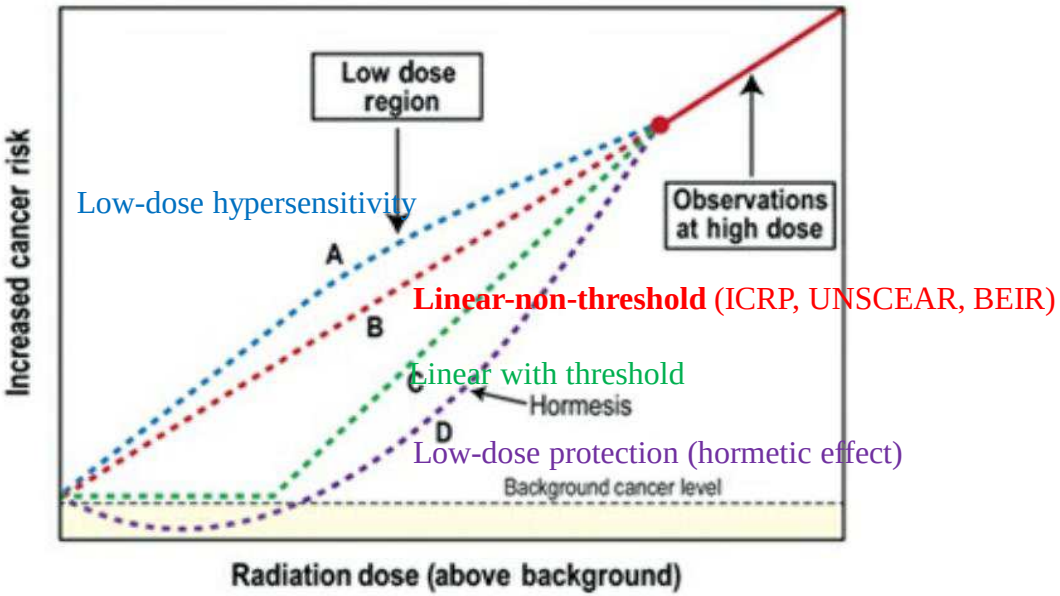
hnRNPA2B1

- A major RNA-binding protein involved in miRNA transport into the EVs
- It is involved in DNA damage response and in altered telomere maintenance



Dose-response relationships

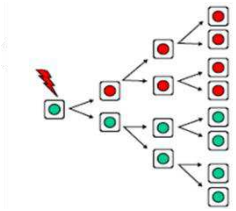


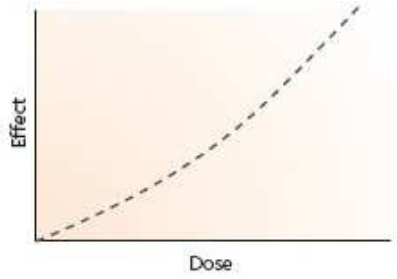


Hall, Garcia – Radiobiology for the Radiobiologist, 8th Edition

DNA-targeted effects

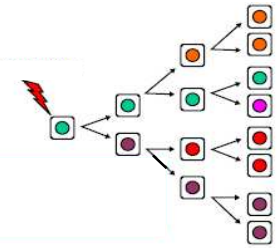
Clonal changes



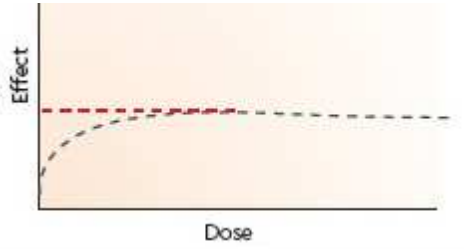


Non-DNA-targeted effects and Non-targeted effects

Non clonal changes



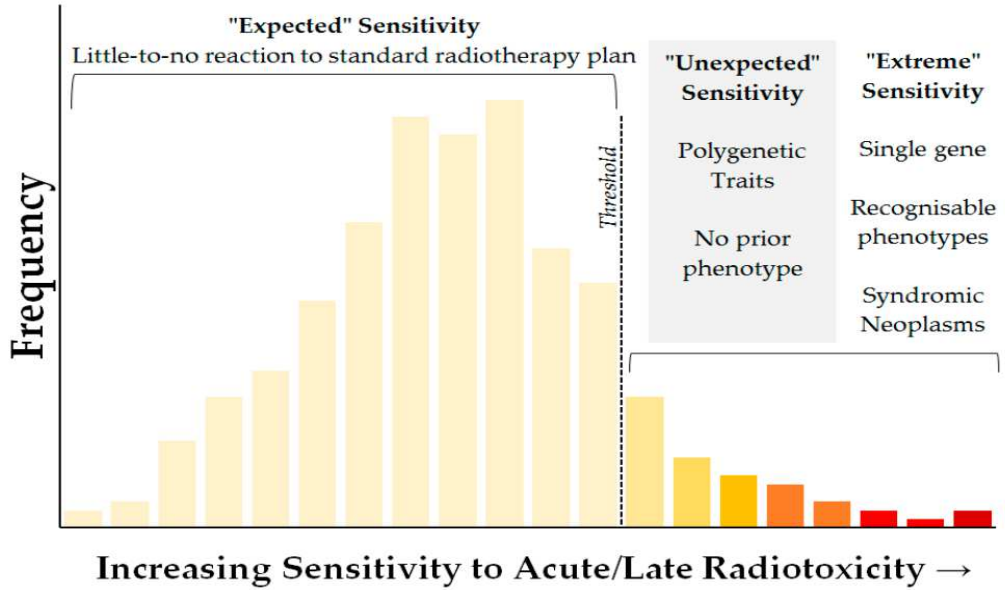
?

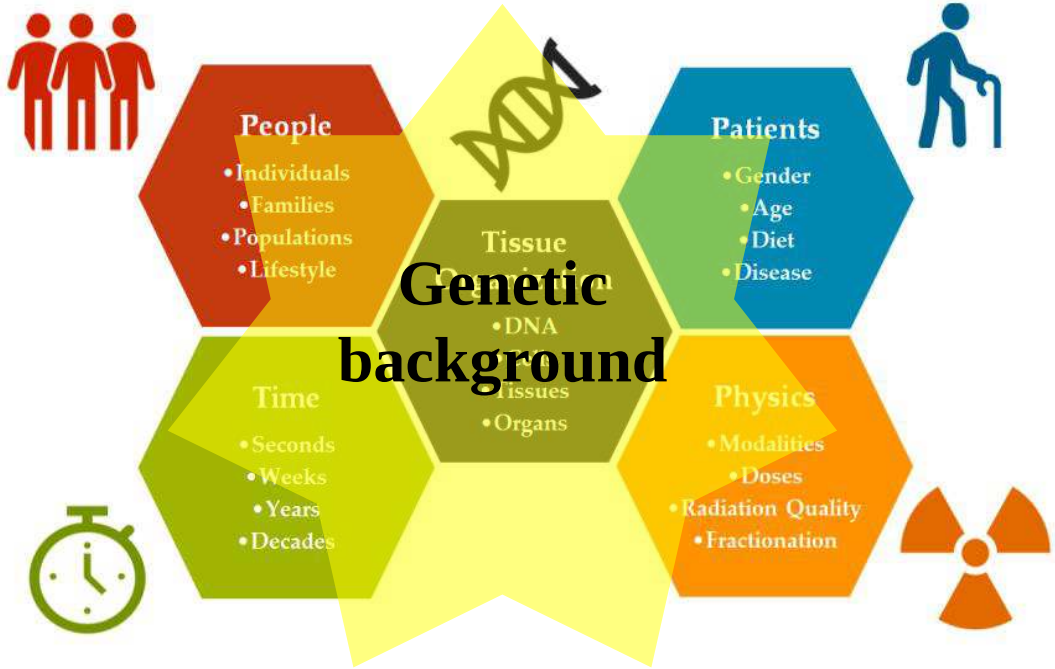


Biological modifiers of radiation effects

Individual radiosensitivity and susceptibility:

(Individual tolerance to ionizing radiation induced toxic effects within the healthy tissues and the probability to develop cancer after exposure to a given dose of radiation)

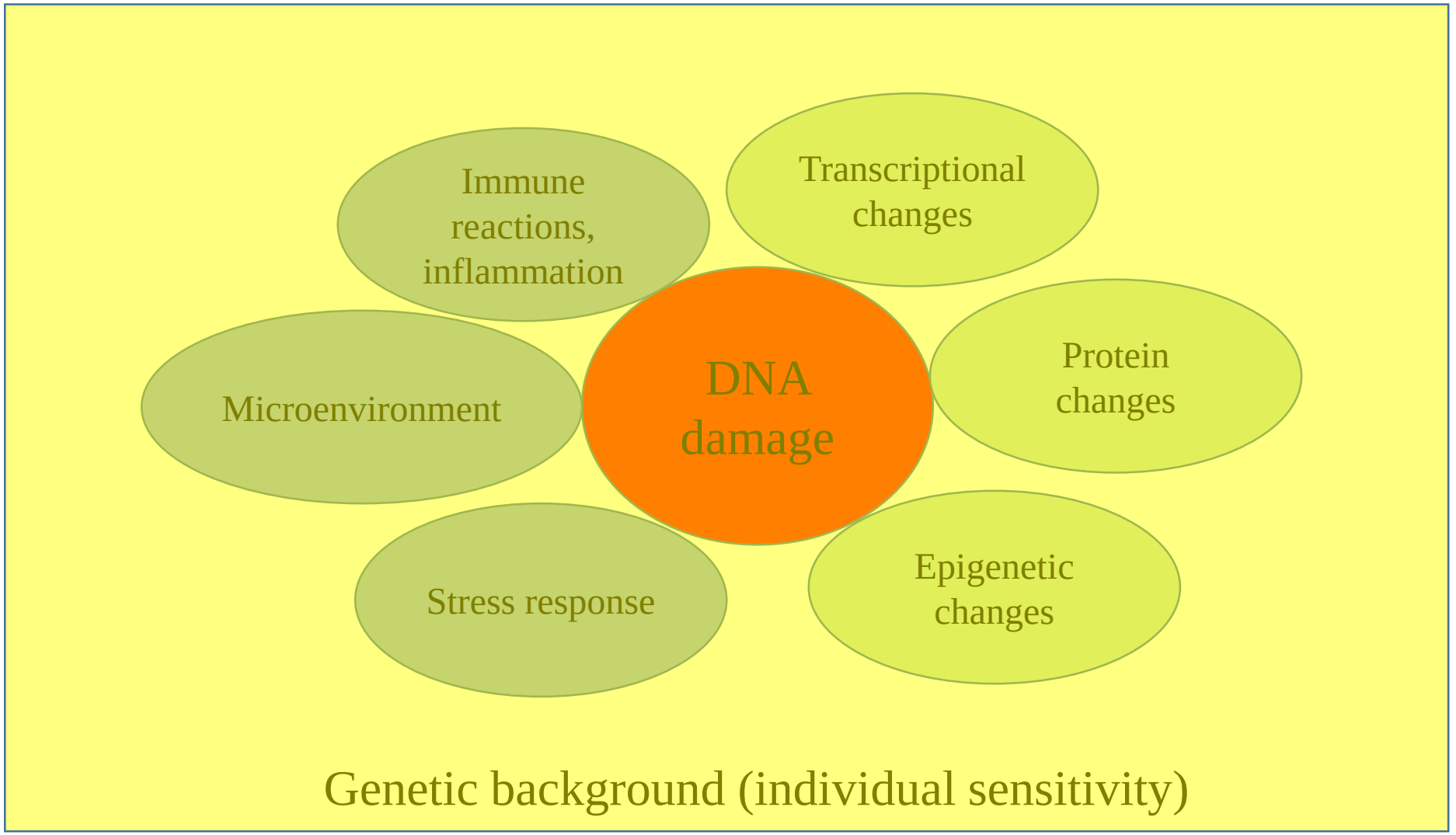




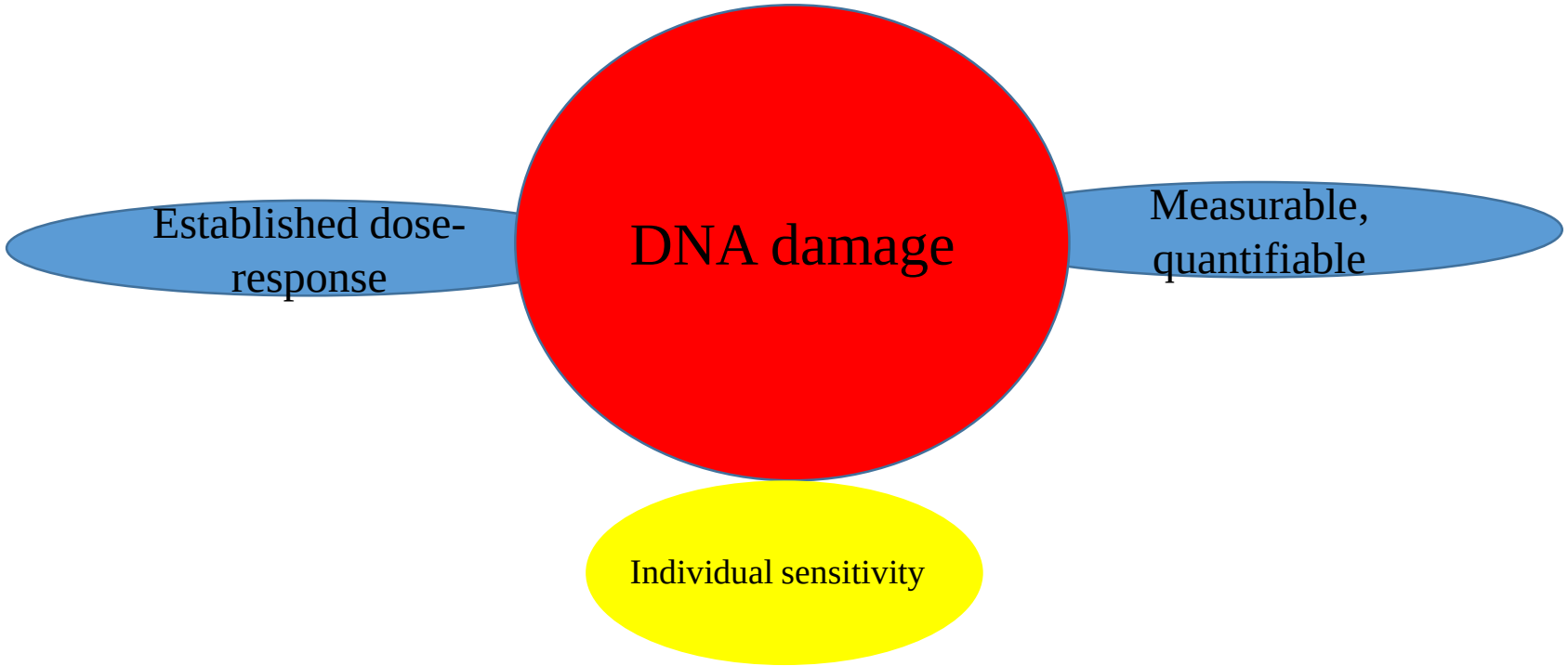
Polygenic!

Habash et al. Cancers 2017

Overall radiation damage



Risk assessment of overall radiation damage in radiation protection



Thank you for your attention!