Akademie věd České republiky

BIOLOGICKÉ ÚČINKY NÍZKÝCH DÁVEK ZÁŘENÍ A JEJICH VÝZNAM PRO RADIAČNÍ OCHRANU

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Oddělení dozimetrie záření
Ústav jaderné fyziky, v.v.i.
Akademie věd České republiky

ÚJF

Zdravotní rizika

Deterministické účinky (tkáňové reakce) mají charakteristické klinické projevy.

Stochastické účinky - tj. zhoubné nádory a změny dědičné se klinickým obrazem neliší od obdobných spontánně se vyskytujících projevů.
Nenádorová onemocnění :

- Choroby srdce, mozková mrtvice, onemocnění zažívacího a dýchacího ústrojí
- Nejistota tvaru závislosti dávka-účinek v oblasti nízkých dávek
- Buněčné a tkáňové mechanismy pro vznik těchto onemocnění nejsou známy
- Dostupná data nedovolují jejich začlenění do hodnocení újmy po ozáření nízkými dávkami přibližně pod 100 mSv

Excess relative risk

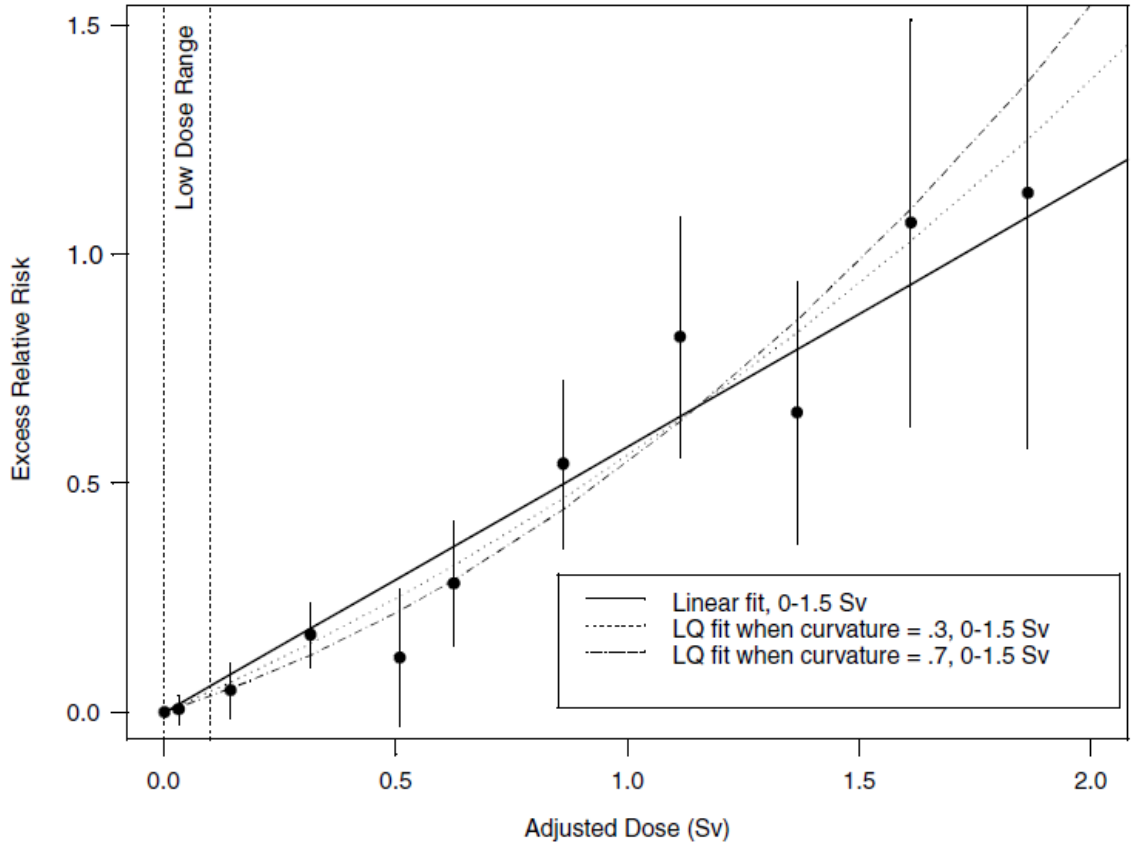
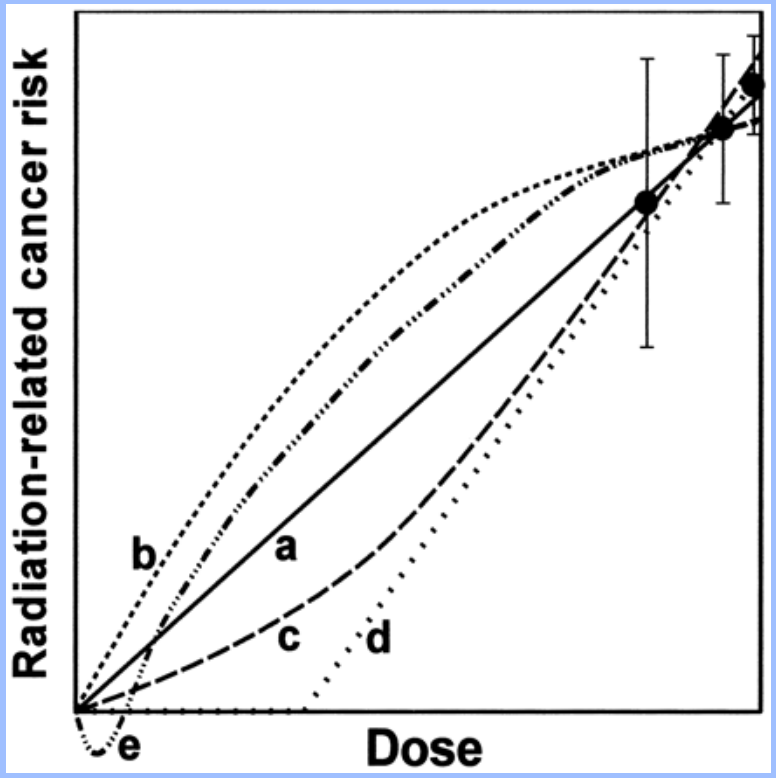


FIGURE 10-2 Illustration of LSS DDREF. Plotted points are the estimated ERRs for solid cancer incidence (averaged over sex, for individuals exposed at age 30 at attained age 60) from LSS subjects in each of 11 dose categories. The vertical lines extend two standard errors above and below the estimates. The solid line is a linear fit to the data for dose range 0–1.5 Sv, with slope $\alpha_L = 0.56$. The other two curves are estimated LQ models for the same dose range, when the curvature, θ, is constrained to be 0.3 Sv^{-1} (resulting in estimated linear coefficient $\alpha_{LQ} = 0.43$) and 0.7 Sv^{-1} (resulting in estimated linear coefficient $\alpha_{LQ} = 0.32$). The LSS DDREFs that result from these are 0.56 / 0.43 = 1.3 and 0.56 / 0.32 = 1.8, respectively.

Health risks from exposure to low levels of ionizing radiation, BEIR VII-Phase 2, 2006

Dose-effect relation

the shape of the dose-response relationship at low doses and low dose rates for radiation-induced health effects, particularly cancer, are critical judgements for radiation protection policy and risk assessment. In brief, five basic model options on low dose response tend to be considered:



(UNSCEAR 2000; CERRIE 2004; NRC 2006; French Academy 2005; ICRP 2007; Brenner et al. PNAS 100(24), 13761, 2003)

- a) linear-no-threshold,
- b) supra-linear (hypersensitivity),
- c) upwardly curving with no threshold,
- d) linear or upwardly curving but with a zero-effect interval below a given threshold dose, or
- e) more complex bi-modal relationships (including beneficial health effects or hormesis at low doses)

Radiation biology studies – from cell culture to epidemiology

Expectations for radiation biology:
It is possible to extrapolate low dose and low dose effects from high radiation dose studies

Unfortunately this may not be possible

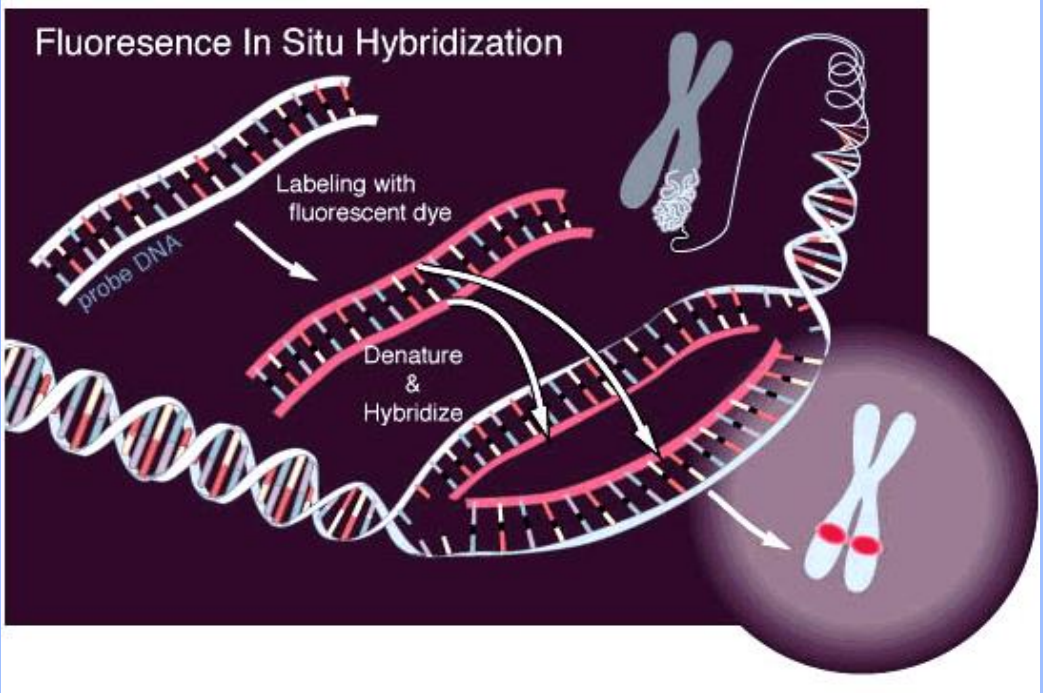
Epidemiological data are often contradictory, mouse data as well

Many studies on mRNA and protein expression show distinctly different patterns of gene expression for moderate and low doses of radiation

Even cell culture studies confirm that this is an erroneous assumption

e.g. In stem cells both hypersensitivity and hormesis

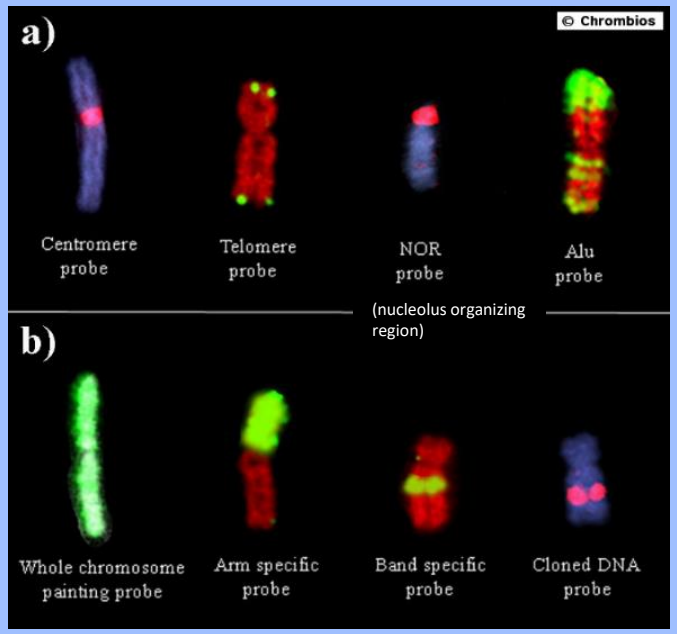
Fluorescence in situ Hybridization (FISH)



The probes: repetitive and "unique" sequences.

Targets:

- Metaphase chromosomes
- Interphase nuclei
- Extended chromatin fibers
- Entire Cells/RNA
- Tissue sections

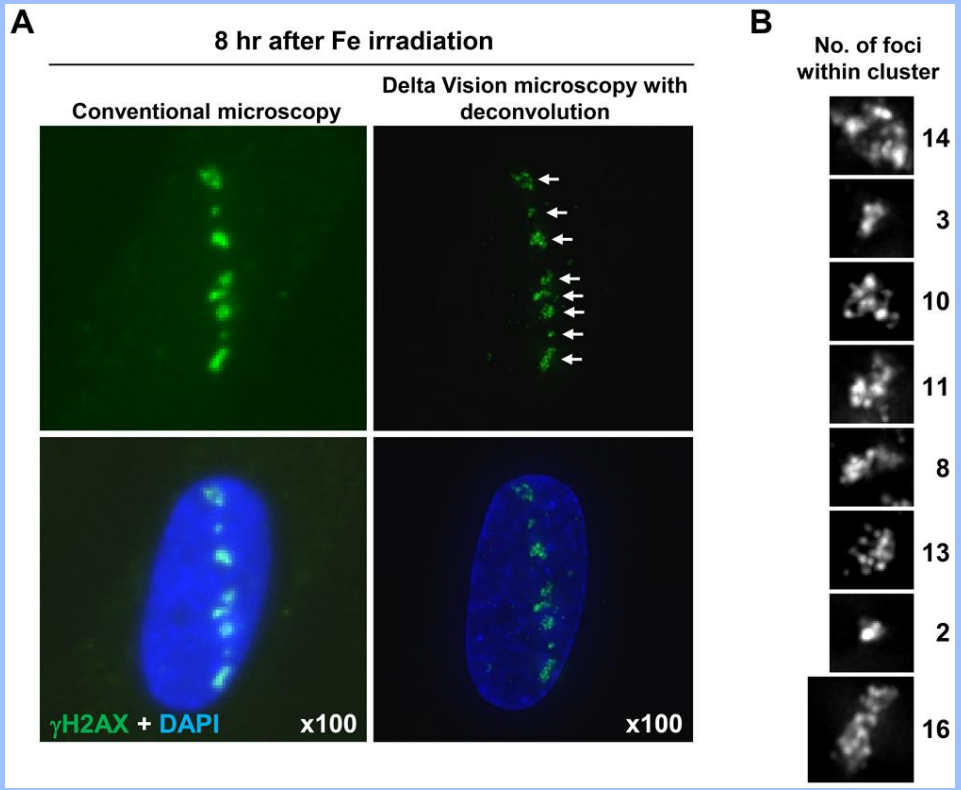


- applies fluorescently labeled DNA probes to detect gene or chromosome abnormalities that are generally beyond the resolution of routine cytogenetics. The DNA is first denatured. The fluorescently labeled probe is added and hybridizes with the sample DNA at the target site as it reanneals back into a double helix. The probe signal is seen by a fluorescent microscope.

γH2AX foci

- H2AX represents 2 – 10% of the H2A subfamily of histone proteins in chromatin
- Phosphorylated rapidly in response to DSB at serine 139
- ~1% of the H2AX phosphorylated per Gy and number of γH2AX foci

  ≈ number of DSB
- Acts as major recruiter of repair enzymes



High resolution microscope analysis revealed clustered γH2AX foci formation within the tracks following Fe irradiation 48BR (WT) primary G0/G1 cells.

Nakajima et al. (2013) PLoS ONE 8(8): e70107

DNA damage and repair

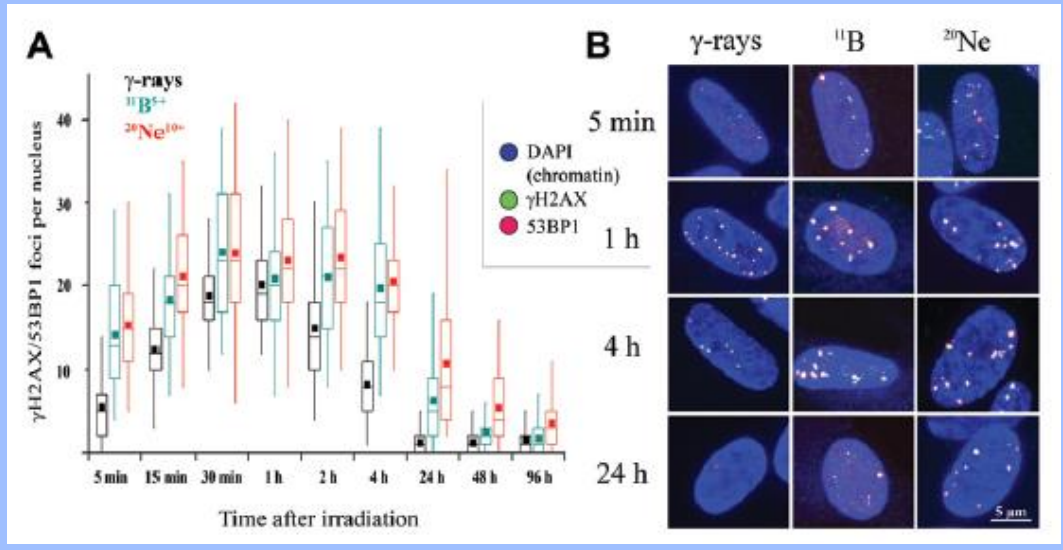


Fig. 5 γH2AX/53BP1 foci formation and loss (DSB repair dynamics) upon exposure to radiation of different qualities. NHDF-Neo cells were irradiated in the perpendicular (90°) geometry with 1 Gy of γ-rays, ^{20}Ne ions (LET = 132.1 keV μm^{-1}, E = 46.6 MeV per n) or ^{11}B ions (LET = 138.1 keV μm^{-1}, E = 8.1 MeV per n) and fixed at different times PI, as indicated. (A) Quantification of the number of γH2AX/53BP1 foci in 3D images. Sham-irradiated cells contained (not shown) 0.1 foci per nucleus on average. The box-and-whisker plot indicates the mean (black square), median (median line inside the box), 25th and 75th percentiles (the top and bottom of box, respectively), and minimum and maximum (whiskers) of the pooled data from two experiments (approximately 100 counted cells). (B) Representative maximum intensity images of the corresponding cell nuclei. γH2AX (green), 53BP1 (red), chromatin (DAPI).

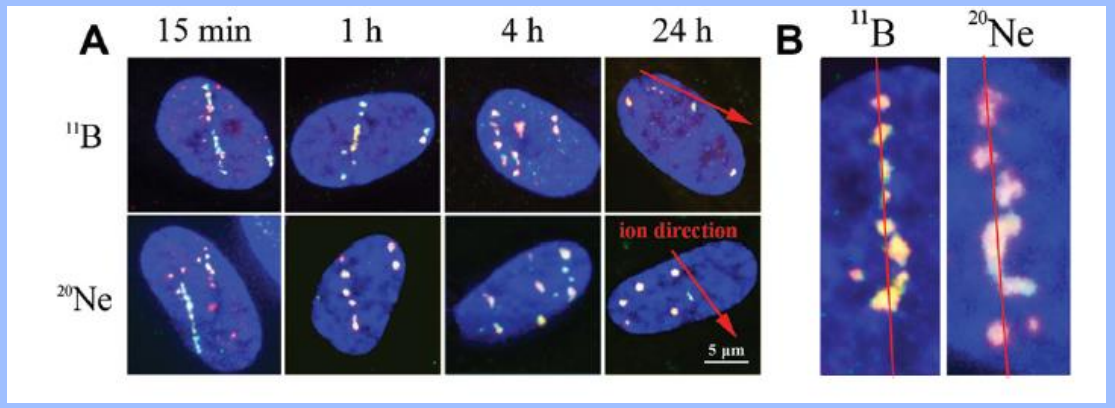
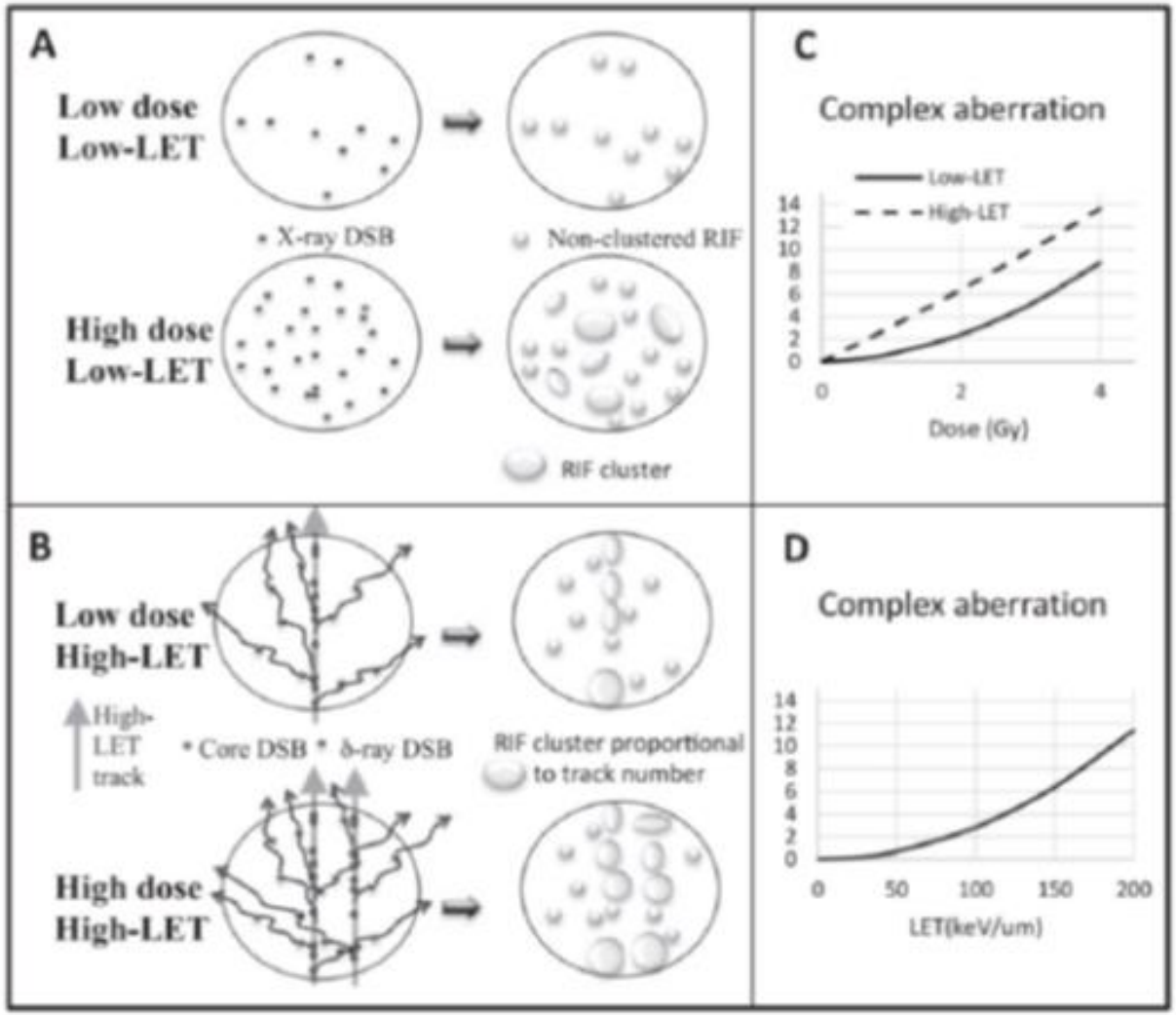


Fig. 6 Structures of γH2AX/53BP1 focus streaks and their dynamic changes with time PI. NHDF-neo cell nuclei were exposed to an average of three ^{20}Ne or ^{11}B ions (*i.e.*, 1.2 and 1.0 Gy, respectively) emitted at a sharp angle to the cell monolayer. Cells were spatially (3D) fixed at the indicated periods of time PI, and immunostaining for γH2AX (green) and 53BP1 (red) repair foci is presented. (A) Comparisons of γH2AX/53BP1 focus streaks induced by boron and neon at the indicated periods PI. (B) Detailed structures and deflections of foci from a linear particle track observed at 2 h after radiation exposure. Maximum images comprising ~25 superimposed 0.25 μm-thick confocal slices are shown in the *x*–*y* plane in both A and B. Chromatin was counterstained with DAPI (blue).

Ježková et al. (2017) Nanoscale, DOI: 10.1039/C7NR06829H

Radiation induced foci



Dose dependence of RIF clustering. Panel A: shows the formation of radiation-induced foci (see ref. 252) from double-strand breaks (DSB) in nuclei exposed to low and high doses of X ray. Panel A shows that RIF clustering would be expected to occur predominantly after high dose. Panel B: A similar representation for low and high doses of high-LET ions showing RIF clustering occurs along the track independent of the dose. Panels C and D: A consequence of clustering is the formation of complex chromosome aberrations, thus it would be expected that complex chromosome aberrations would differ based on dose (panel C) and LET (at a constant fluence) (panel D). Panels C and D: Y-axis are number of complex chromosome aberrations.

Sridharan et al., Radiat. Res. 183, 1, 2015

Biodosimetry Research Team
NIRS, Japan

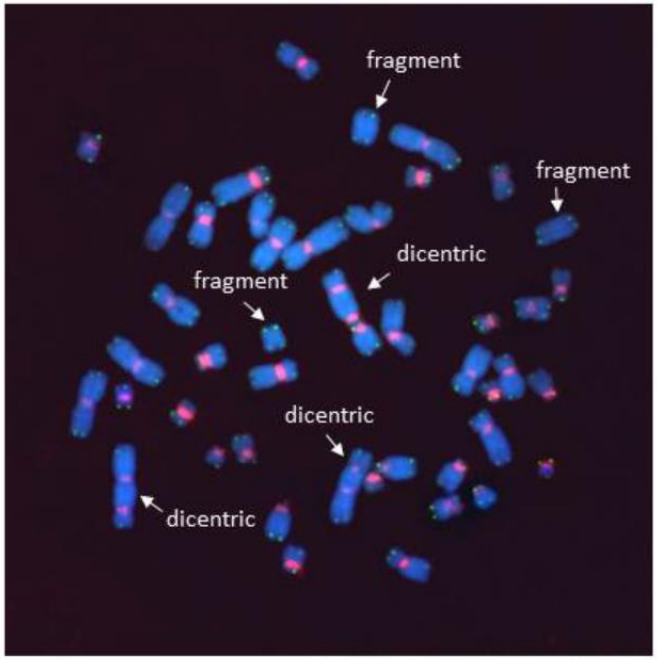


Fig. 24 FISH on gamma-irradiated chromosomes using centromere- and telomere-specific peptide nucleic acid (PNA) probes. Centromeres and telomeres were stained with red and green, respectively. [Cytologia 76: 1-2, 2011, modified]

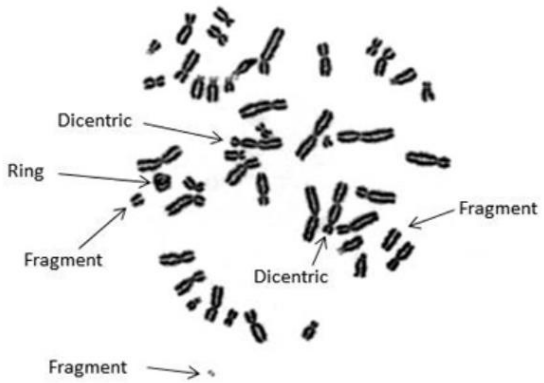
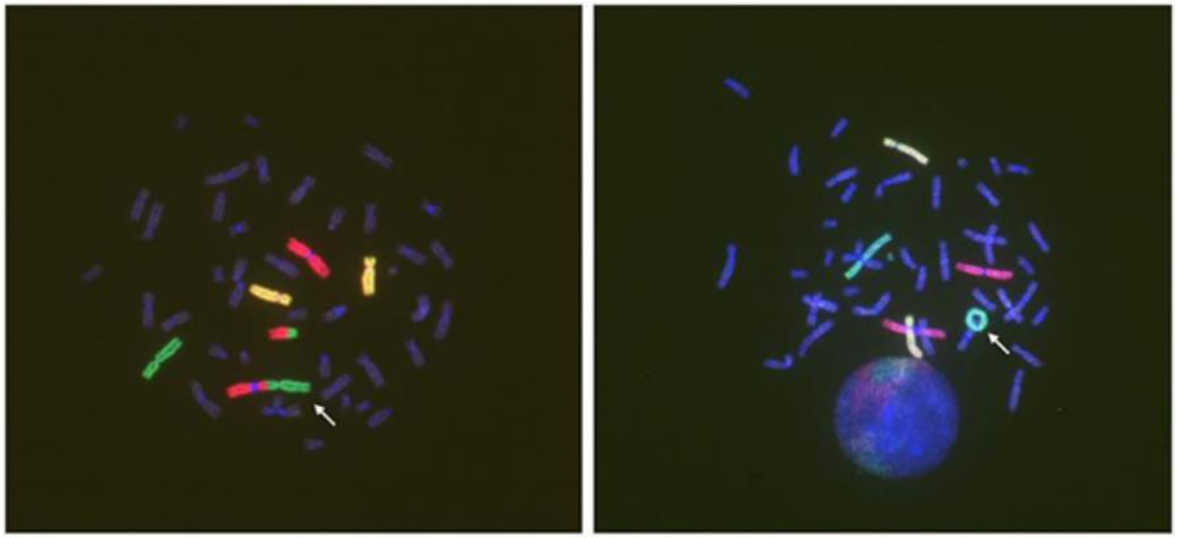


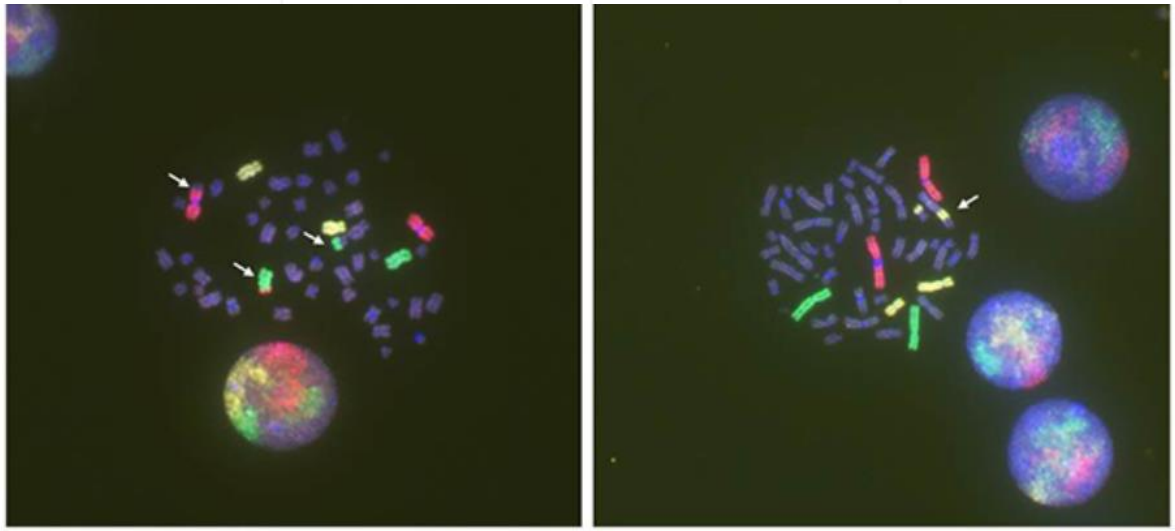
Fig. 17 A human metaphase cell. Peripheral blood lymphocytes were irradiated with ^{60}Co-gamma ray (5 Gy). Dicentric chromosomes, a ring chromosome and fragments were observed.

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



Dicentric (arrow) Ring (arrow)

Fig. 3-2 Chromosomal rearrangements detected by 3-color FISH.



Translocations (arrows) Insertion (arrow)

Fig. 3-3 Chromosomal rearrangements detected by 3-color FISH.

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

DNA mutations and carcinogenesis

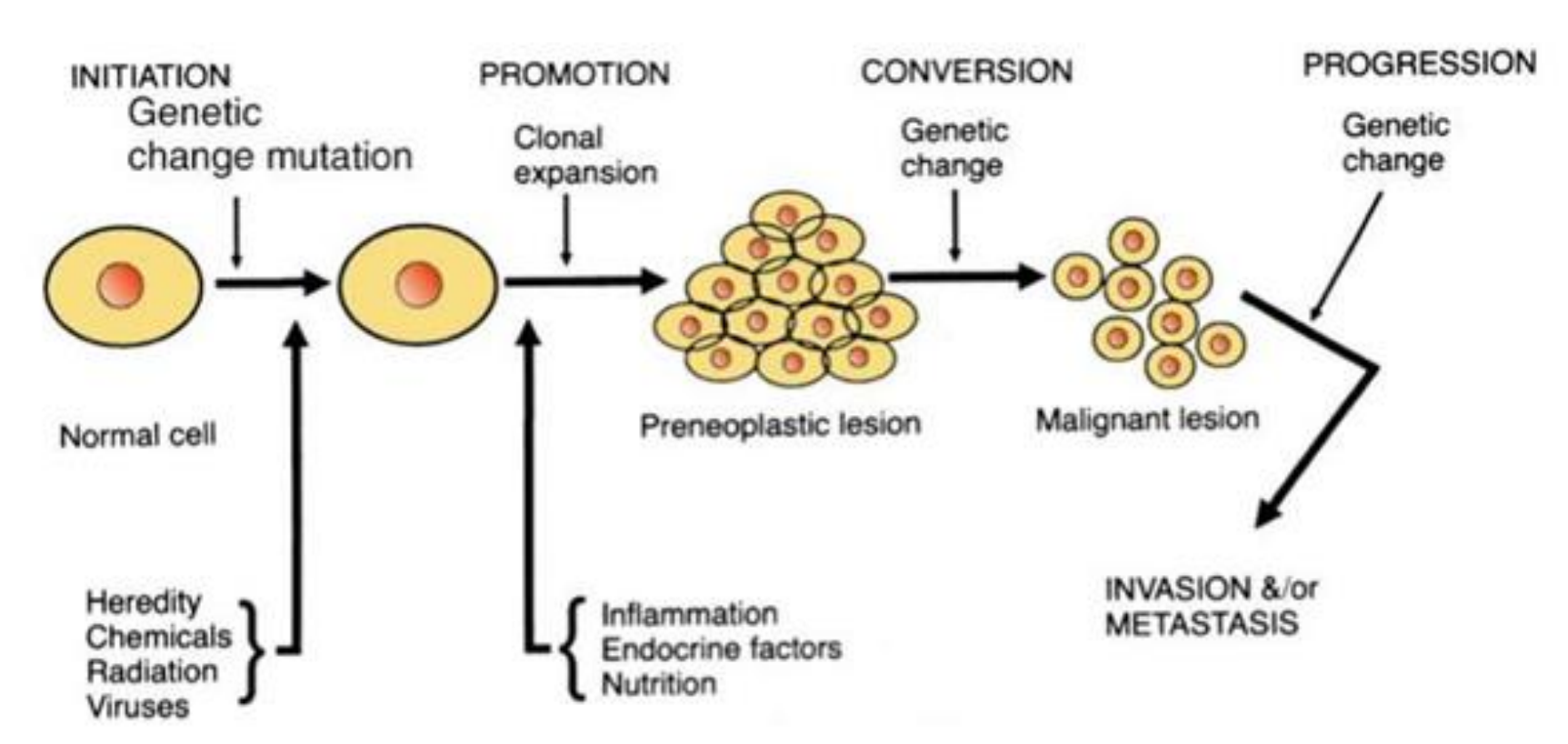
- Each mutation of the somatic cell (especially the stem cell) may be the first step towards carcinogenesis
- At least two mutations are required to produce a neoplastic phenotype, and accumulation of multiple mutations is frequently required

Multistep model of carcinogenesis

Initiation
Activation of oncogenes

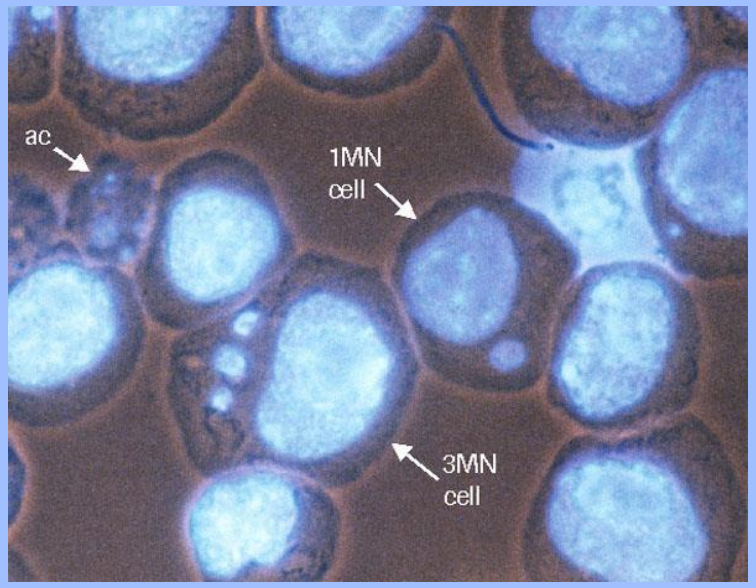
Promotion
Mutation in tumour supressor genes

Progression
Activation of genes that promote metastasis, invasion, angiogenesis, immunological tolerance etc.

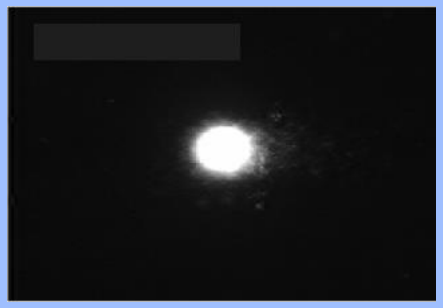


Single cell assays for evaluation of DNA damage

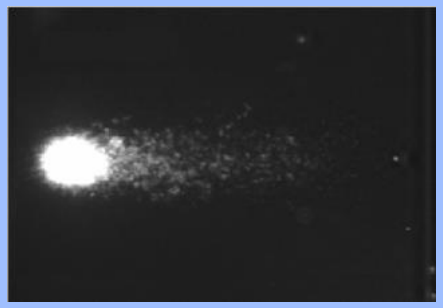
Micronuclei test

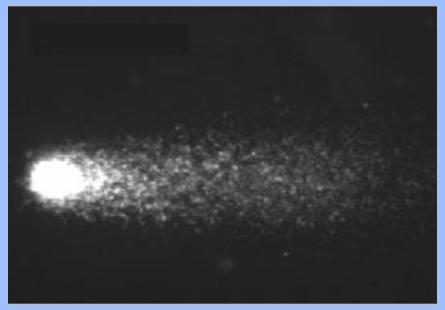


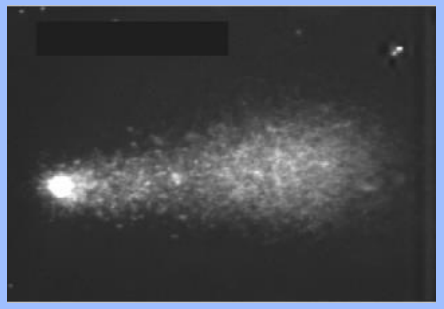
Comet assay



No or low DNA damage.







High and very high DNA damage.

Bystander effects

The central dogma of radiation biology, that biological effects of ionizing radiation are a direct consequence of DNA damage occuring in irradiated cells, has been challenged by observations that genetic/epigenetic changes occur in unexposed “bystander cells” neighboring directly/hit cells, due to cell/to/cell communication or soluble factors released by irradiated cells.
To date, the vast majority of these effects are described in cell-culture systems, **while *in vivo* validation and assessment of biological consequences within an organism remain uncertain**.

Mancuso et al. Curr. Mol. Medicine, 2012

Bystander effects: Cell communication

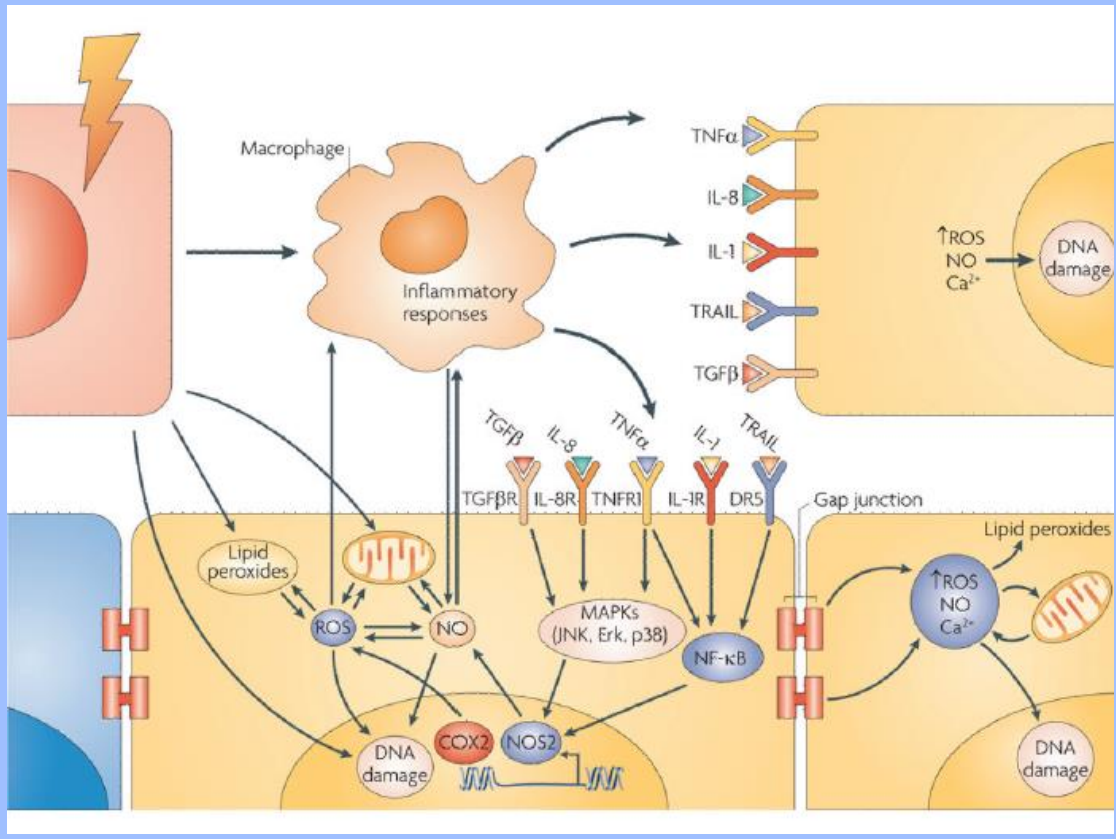
- Through gap junctions and the cytokine signals into the extracellular matrix.
- ***In vivo***: macrophages may be important mediators, which release bystander signals affecting nonirradiated cells. Cytokine-mediated signaling, signal transduction through MAPKs and nuclear factor kB alongside the production of reactive oxygen and nitrogen species.

Intracellular signalling molecules

p53 tumour protein 53
CDKN1A cyclin-dependent kinase inhibitor
MAPK mitogen-activated protein kinases
ATR ataxia telangiectasis and Rad3 related
DNA-PK DNA-dependent protein kinase
PKC Protein kinase C
ATM ataxia telangiectasia mutated protein

Intercellular signalling molecules

ROS - reactive oxygen species
RNS - reactive nitrogen species
NO - nitric oxide
TGFβ1 transforming growth factor β cytokines
IL 8 interleukin
TNFα - tumor necrosis factor α

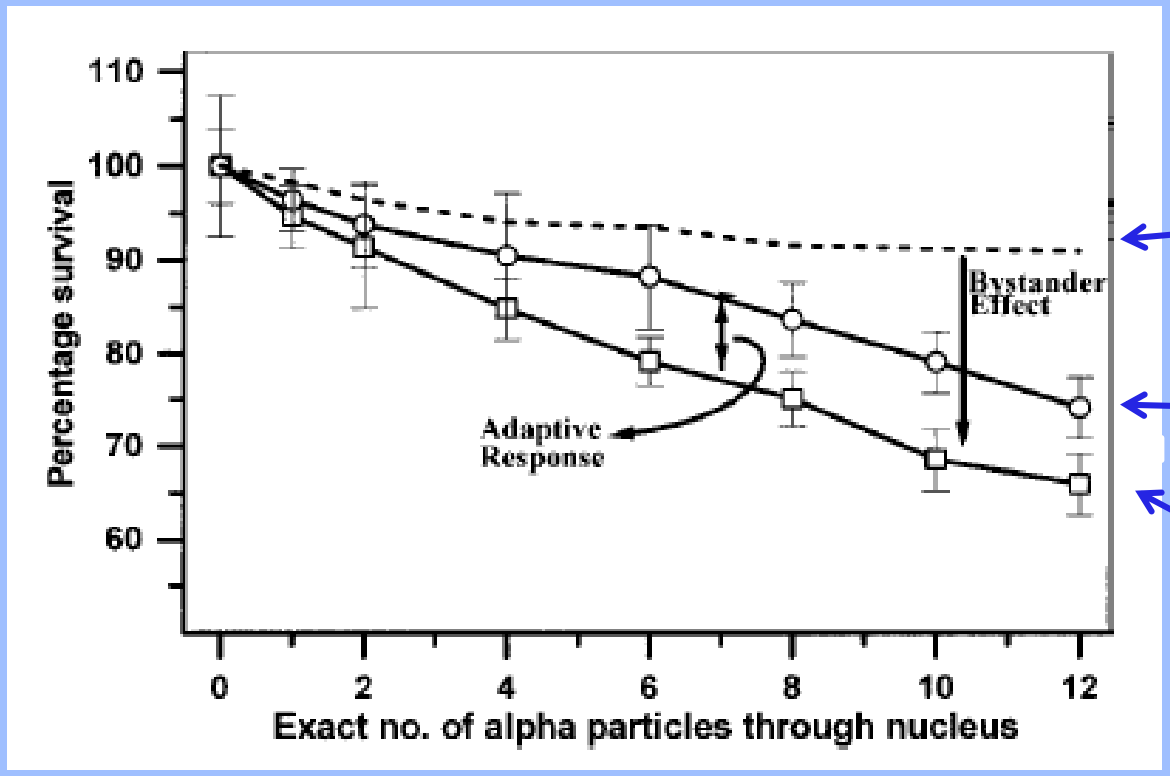


Prise and O´Sullivan, Nat. Rev. Cancer 9, 351, 2009

Non-target theories: Adaptive response

- small doses of radiation (1-100 mGy of gamma rays) induce protect mechanisms against following challenging higher doses (decline above 200 mGy, disappear above 500 mGy)
- these mechanisms are not induced with lower doses or without radiation
- small doses can have beneficial effect probably due to enhanced DNA repair ability and cellular antioxidant activity (possibly constitute a complementary defense mechanism to apoptosis)
- Cells, animals, humans (different endpoints: chromosomal aberrations, micronuclei formation, gene mutations, cell killing)

Adaptive response + Bystander effect



Prediction by linear non-threshold model

6 hours prior to alpha 20 mGy of gamma → adaptive response

Reality: decrease of survival compared to linear model due to bystander effect

Sawant, S.G. et al. (2001) Radiation Research 155(3), 397-401

Non-target theories: Abscopal effects

- First time mentioned in Mole, R. Whole body irradiation therapy – radiobiology or medicine? Br J Radiol. 234–241, 1953
- in oncology, the abscopal effect refers to the ability of localized radiation to trigger systemic antitumor effects (tumor regression at a site distant from the primary site of radiotherapy)
- the immune system is a major determinant in regulation of abscopal effects

Non-target theories: Abscopal effects

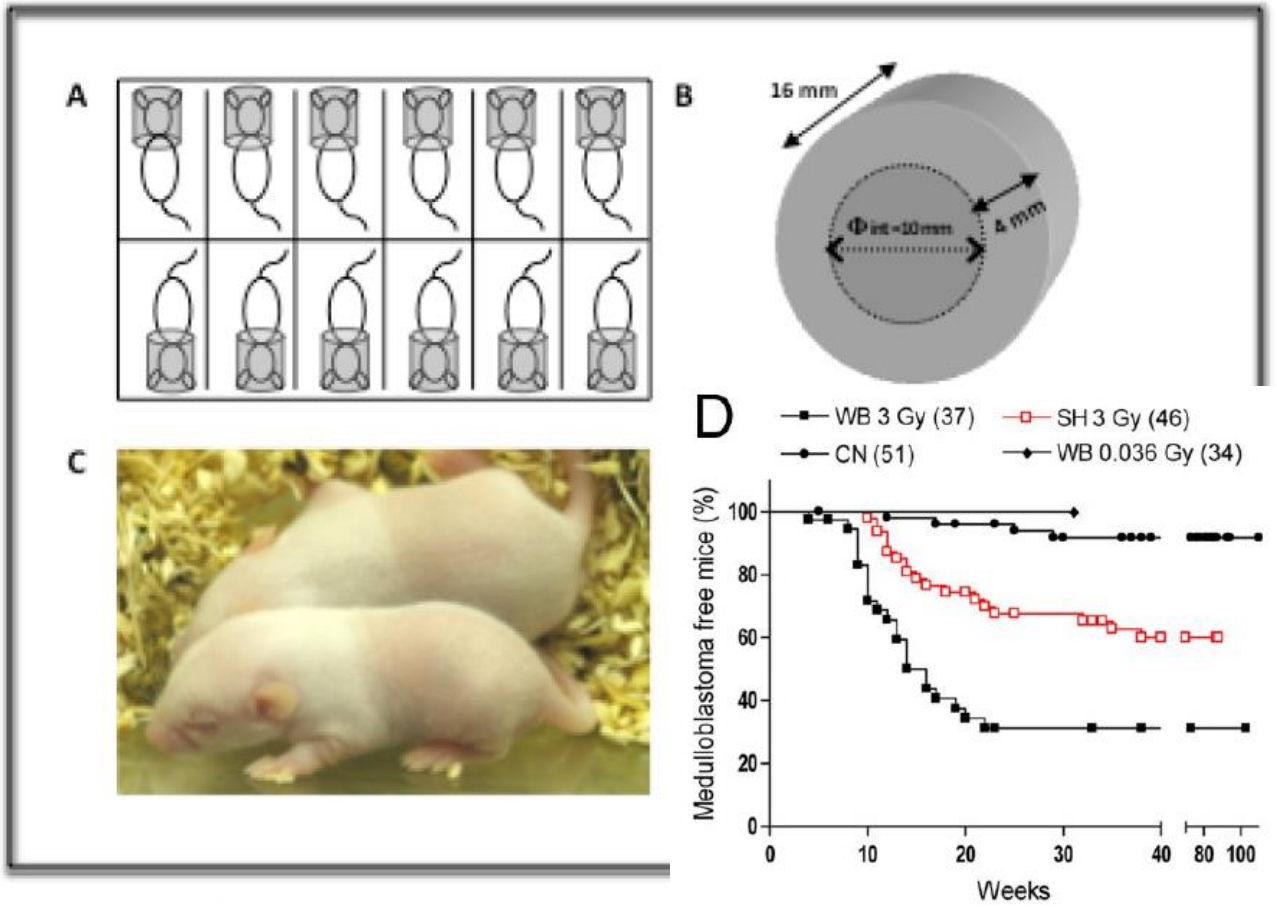


Fig. (2). (**A**) Neonatal Ptch1$^{+/-}$ mice were whole-body (WB) exposed, or irradiated with individual cylindrical lead shields providing protection of heads (SH). (**B**) Demarcation between exposed and shielded regions at postnatal day 10 due to hair-growth delay in exposed skin. (**C**) Characteristics of the lead shields. (**D**) Kaplan–Meier kinetic analysis of medulloblastoma in whole-body irradiated (WB), shield-irradiated (SH), control (CN), and Ptch1$^{+/-}$ mice exposed to 0.036 Gy (WB-0.036), the scatter dose to the shielded cerebellum. Modified from ref. [88], *PNAS* August 26, 2008 vol. 105 no. 34 12445-12450, Copyright 2008 National Academy of Sciences, U.S.A.

Mancuso et al. Curr. Mol. Medicine, 2012

Bystander/Abscopal effects in mammalian systems

Bystander responses predominate at low doses of relevance to radiation risk analysis (< 0.2 Gy) and therefore need to be fully characterized. Nevertheless, there is a general lack of in vivo data for bystander/abscopal responses in exposed individuals. Therefore, the search of the effect *in vivo* in mammalian systems represents a priority in the study of cancer risk from low-dose radiation, not only for environmental and occupational exposures but for clinically relevant dose and dose distributions at tissue and whole-body level.

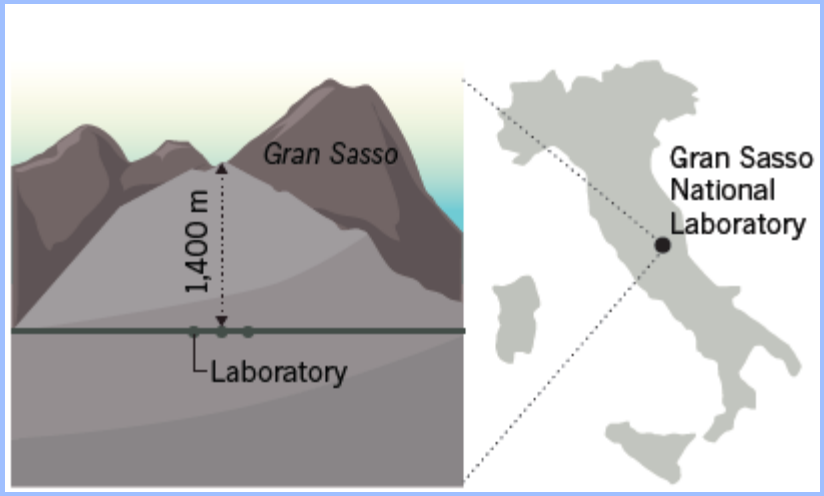
Mancuso et al. Curr. Mol. Medicine, 2012

There results represents the first proof-of-principle that bystander effects are factual *in vivo* events with carcinogenic potential, and implicate the need for re-evaluation of approached currently used to estimate radiation-associated health risks.

Mancuso et al. PNAS 105, 12445, 2008

Cell response to extremely low levels of IR

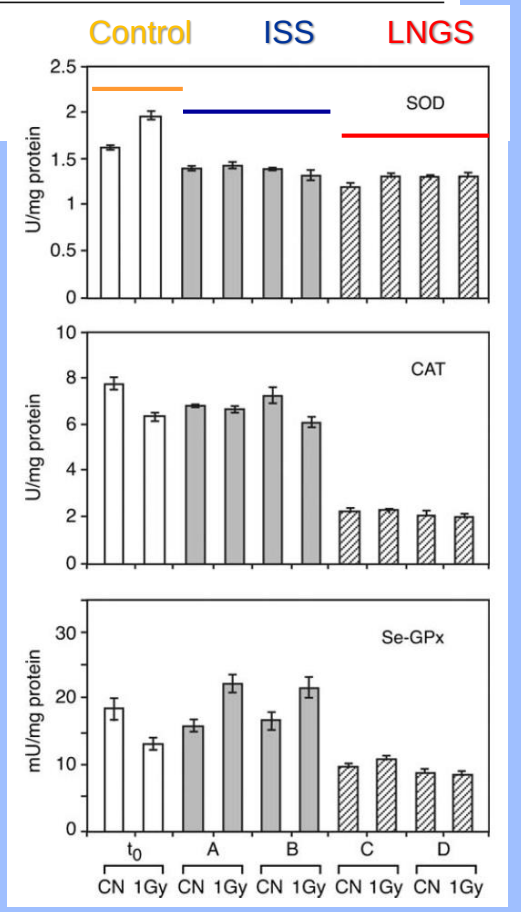
Gran Sasso National Laboratory INFN, Italy



Adopted from Nature 485, 435, 2012

Table 1 Dosimetry estimates

Background radiation component	Reduced background (underground) (nGy/h)	Normal background (external) (nGy/h)
^{222}Rn and daughters[a]	0.17	1.7
All γ-rays[b]	3.6	300
Cosmic rays	Negligible	30[c]
Total dose-rate	3.8	331.7

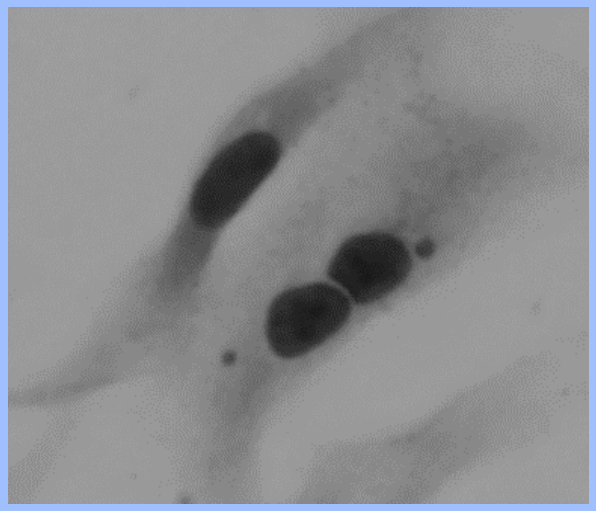


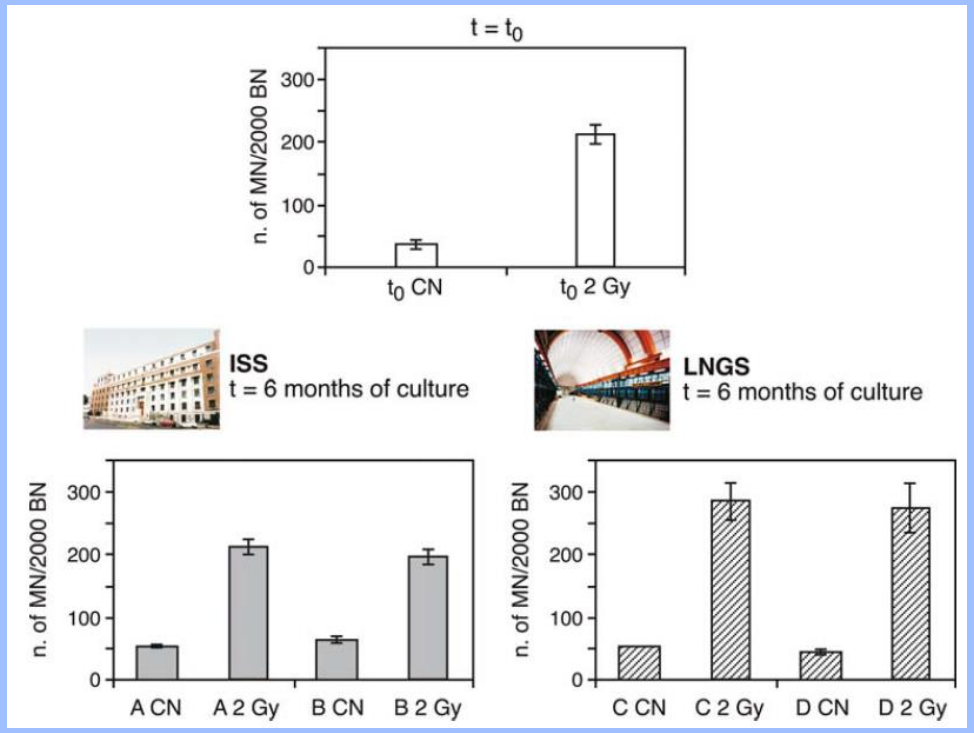
- decrease of antioxidant enzymatic activity after 6 months growth at the ISS and LNGS:

- SOD - superoxide dismutase
- CAT - catalase
- SE-GPx - selenium-dependent glutathione peroxidase

Carbone et al. Radiat. Environ Biophys. 48, 189, 2009

LNGS vs ISS – human lymphoblastoid TK6 cells





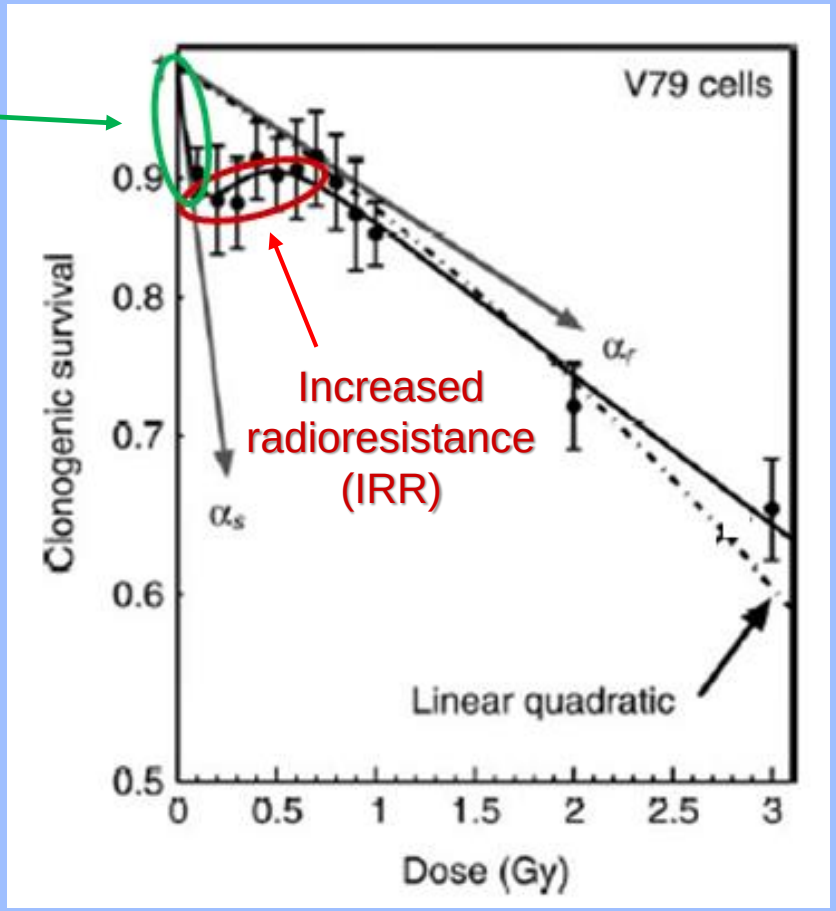
- increase in micronuclei formation after 2 Gy X-rays

→ cells cultivated at extremely low radiation background lose their ability to deal with DNA damage

Carbone et al. Radiat. Environ Biophys. 48, 189, 2009

Hyper-radio-sensitivity (HRS) phenomenon

Hyper-radiosensitivity (HRS)



Marples, Cancer Metastasis Rev 2004

- Low-dose hypersensitivity (up to 300 mGy)
- Induced radioresistance (at higher doses)

- does not exist with neutrons
- shown in vivo with skin, kidney and lung in fractionated therapy with very low applied doses
- after conditioning dose (bellow 300 mGy) effect disappears: adaptive mechanism (possibly associated with amount and rate of DNA repair ?)

Rizika nízkých dávek záření

Rozdíly v odezvě na nízké a vysoké dávky

Nízké dávky < 0.2 Sv	Vysoké dávky > 0.2 Sv
Nízká úroveň buněčné smrti	Vysoká úroveň buněčné smrti
Poškození DNA malé (nebo nedekovatelné)	Poškození DNA značné
Exprese genů	Exprese genů (včetně poškození?)
Epigenetické efekty	Epigenetické efekty
Nízká koncentrace oxidativních radikálů	Vysoká koncentrace oxidativních radikálů
Nepřímý účinek	Přímý účinek
Selektivní apoptóza	Apoptóza (zvýšená úroveň)
Četnost mutací	Četnost mutací
Buněčná transformace	Buněčná transformace
Imunitní odezva (+)	Imunitní odezva (-)
Karcinogeneze (??? %/mSv)	Karcinogeneze (5%/Sv)

Perspectives:

Follow-up of modest changes in gene expressions and enzyme activities

- Gene expression changes
  - ✓ Microarrays
  - ✓ Next-gen sequencing
  - ✓ …
- Epigenetic changes
  - ✓ Micro RNA
  - ✓ Histone modifications
  - ✓ ….
- OMICS approaches
  - ✓ Metabolomics
  - ✓ Melalomics
  - ✓ ….

Děkuji za pozornost