RADIATION DAMAGE SIGNALLING

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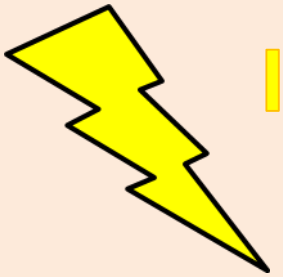
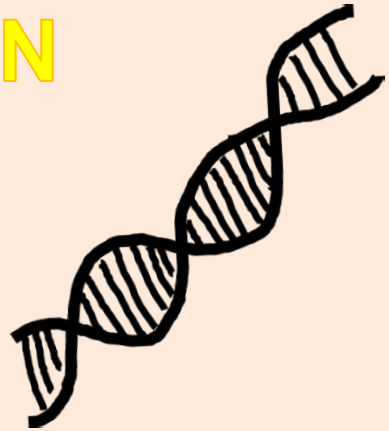
1. DAMAGE RESPONSE (DDR)



IONIZING RADIATION



DNA DAMAGE

DAMAGE RESPONSE (**DDR**) THROUGH **SIGNALLING** AND **REPAIRING** **PATHWAYS** MEDIATED BY

PROTEINS

SENSORS

MEDIATORS

EFFECTORS

(1)

2. SENSING PROTEINS

Protein complexes that **bind to DNA damage sites:**

- **Rad17-RFC-9-1-1 supercomplex**
- **MNR (Mre11-Rad50-NBS1) supercomplex**
- **ku70-ku80 complex**
- **ATRIP complex**

(1) (2)

3. MEDIATOR PROTEINS

Proteins that **bind to sensor complexes to activate themselves and phosphorylate the H2AX histone:**

- **Ataxia telangiectasia mutated protein (ATM)**
- **Ataxia telangiectasia related protein (ATR)**
- **DNA dependent protein kinase (DNA-PKcs)**
- **H2AX phosphorylated histone (γH2AX)**

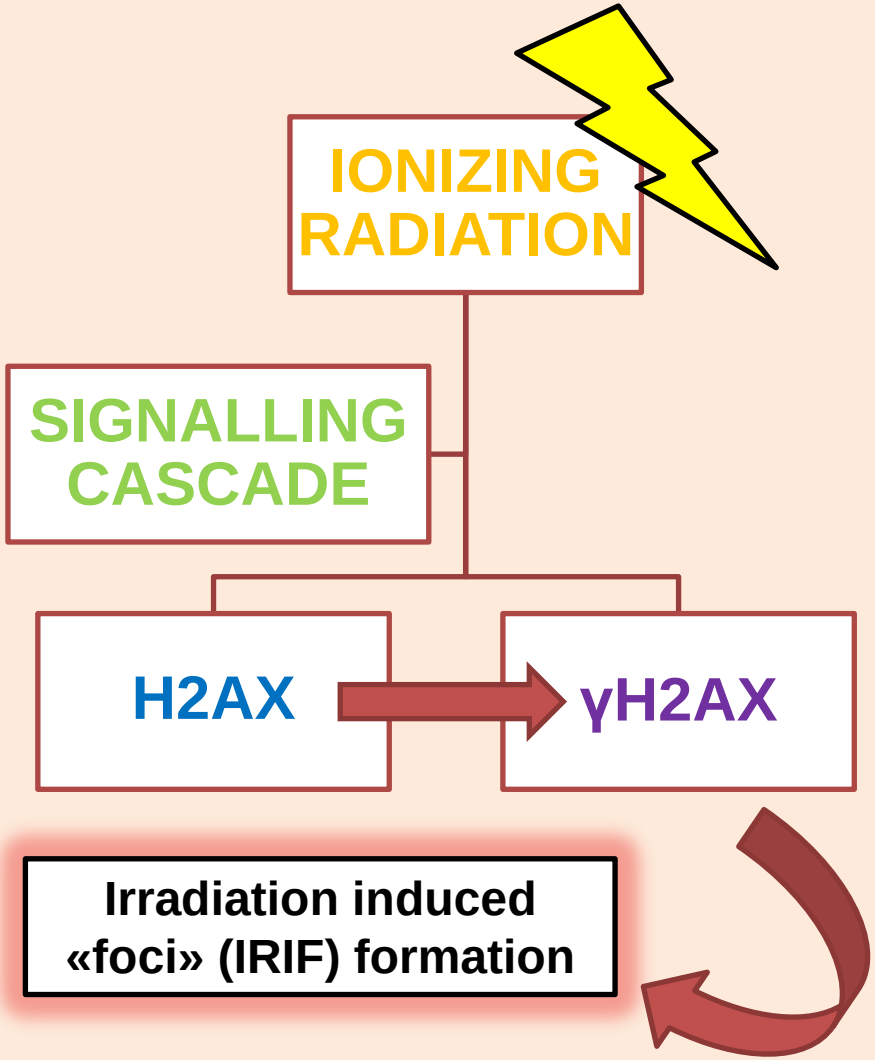
(1) (2)

γH2AX HISTONE

Between 2,4 - 25 % of H2A histones are H2AX

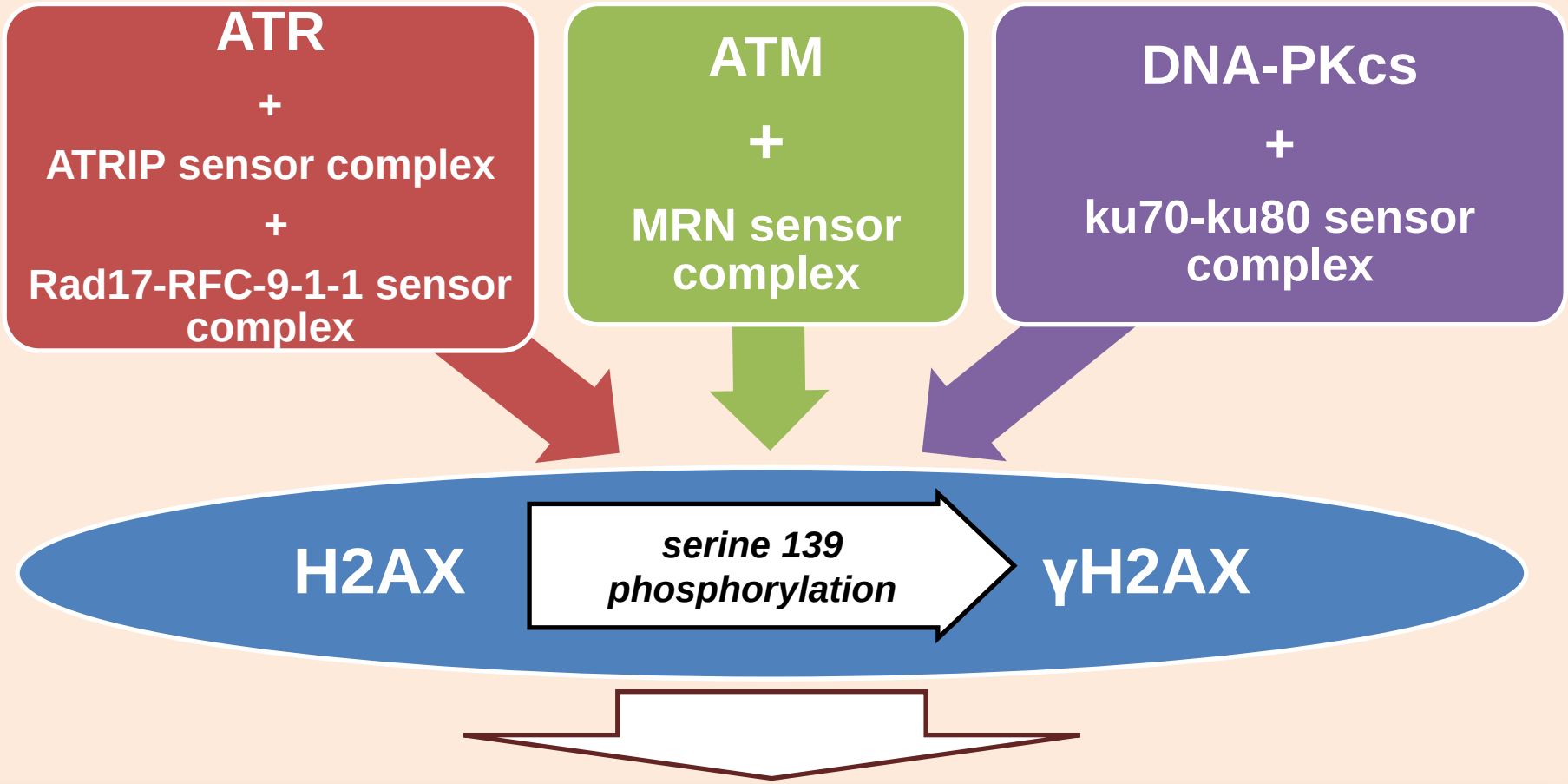
Figure 1. Representation of a nucleosome = DNA + histone octamer (H2A, H2B, H3 and H4)

Source: RCSB Protein Data Bank



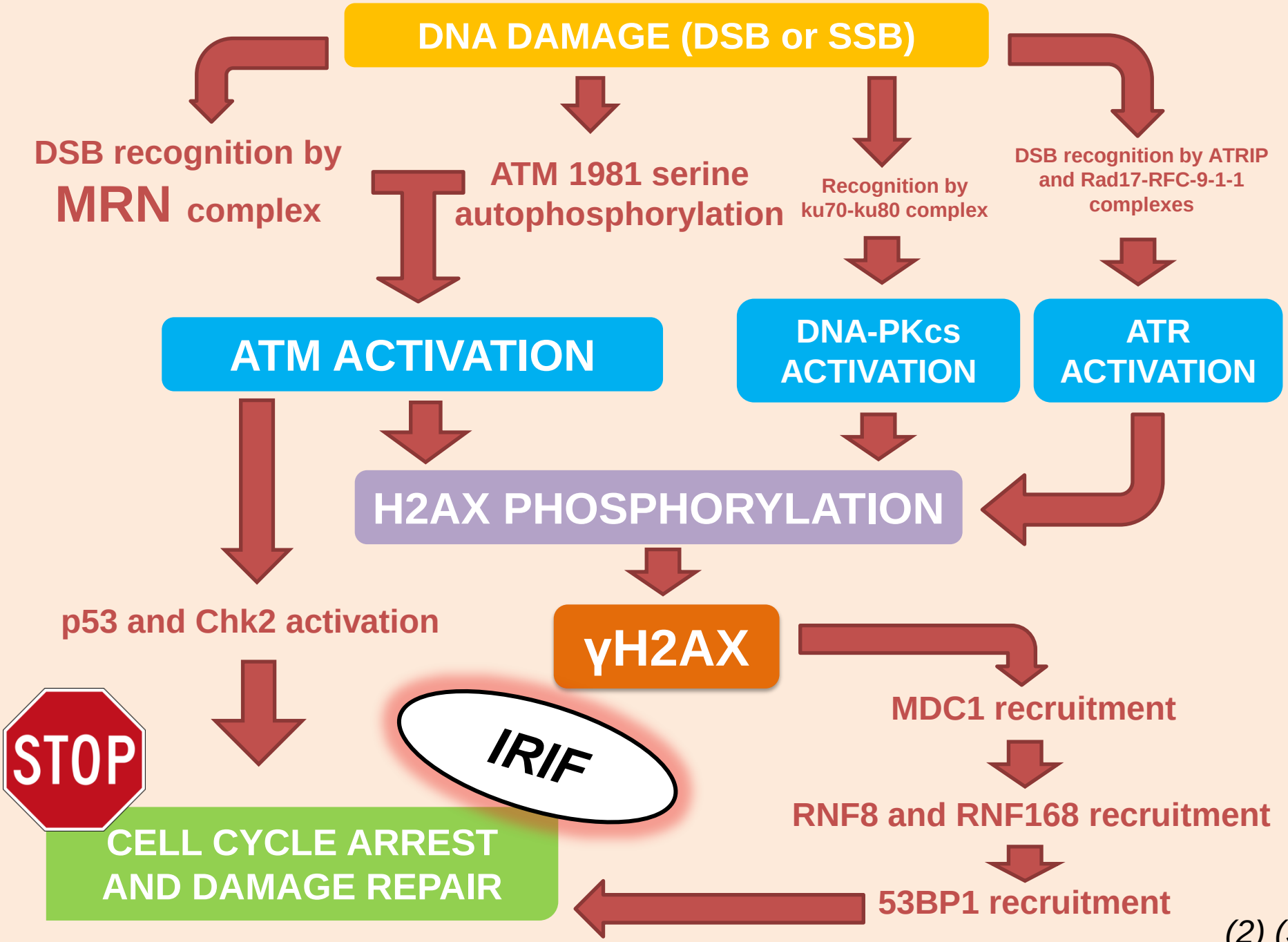
(1) (3)

4. SIGNALLING CASCADE



Irradiation inducen «Foci» (IRIF)

(1) (3)


DNA DAMAGE (DSB or SSB)
DSB recognition by MRN complex
ATM 1981 serine autophosphorylation
Recognition by ku70-ku80 complex
DSB recognition by ATRIP and Rad17-RFC-9-1-1 complexes
ATM ACTIVATION
DNA-PKcs ACTIVATION
ATR ACTIVATION
H2AX PHOSPHORYLATION
p53 and Chk2 activation
γH2AX
IRIF
MDC1 recruitment
RNF8 and RNF168 recruitment
53BP1 recruitment
STOP
CELL CYCLE ARREST AND DAMAGE REPAIR

(2) (3)

5. CLINICAL IMPLICATIONS

5.1. Autosomal recessive congenital disorders:

- **Nijmegen breakage syndrome by mutation of NBS1 gen (8q21-q24)**
- **Ataxia telangiectasia syndrome (or Louis-Barr syndrome) by mutation of ATM gen (11q22-23)**

RADIOSENSITIVE INDIVIDUALS

INCREASED SENSITIVITY TO IONIZING RADIATION

INCREASED CANCER INCIDENCE

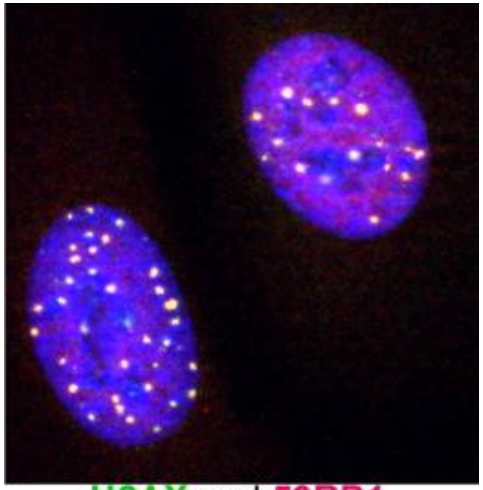
(3) (4)

5.2. Molecular Biology techniques for IRIF visualization and quantification after radiation exposure:

- **Immunofluorescent staining with specific γH2AX antibodies**
- **Immunofluorescent staining with specific 53BP1 antibodies**
- **Flow cytometry**
- **Western Blot**
- **Others**

Figure 2. Tumor cells irradiated with 10 Gy, stained by immunofluorescent techniques and visualized 24 hours thereafter by confocal microscopy. The γH2AX And 53BP1 colocalication at IRIF is shown.

Source: Sak A, Stuschke M. Use of γH2AX and other biomarkers of double-strand breaks during radiotherapy. Semin Radiat Oncol. 2010 Oct;20(4):223-31.



H2AX and 53BP1

DUE TO THE HIGH γH2AX CONCENTRATION IN PHASE G1 IN COMPARISON WITH THE PHASE S γH2AX CONCENTRATION, THE CELL CYCLE PHASE IN WHICH THE CELLS ARE ANALYSED MUST BE TAKEN INTO ACCOUNT!!!

(3) (4)

5.3. Utility of IRIF as a biomarker for radiosensitivity:

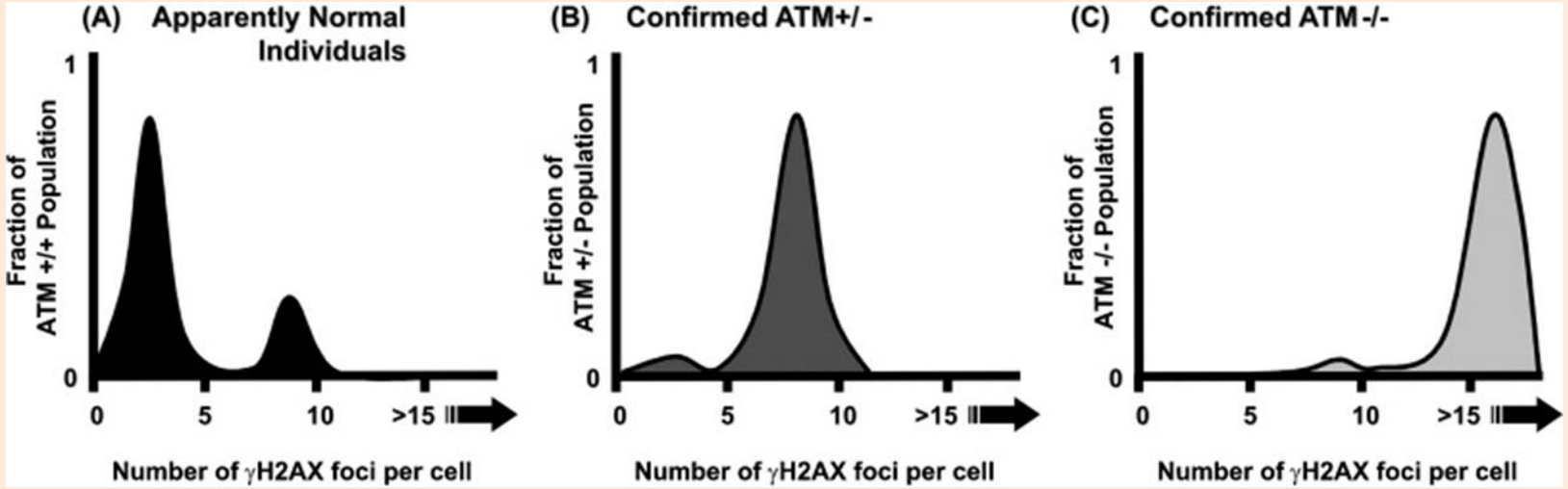
- Knowing the tissular damage after radiotherapy (biological dosimetry after ionizing radiation exposure *in vivo*)
- Knowing the level of reparation after irradiaton
- Diagnosis of human syndromes with associated radiation sensitivity
- Identification of impacting polymorphisms or asymptomatic individuals with DSB repair defects
- Identification of heterozygotes for DDR proteins or individuals with mildly impacting polymorphic changes

EVALUATION OF RADIOSENSITIZER AND RADIOPROTECTOR DRUGS EFFECTIVENESS

OPTIMIZATION OF RADIOTHERAPY IN EACH PATIENT

(3) (4)

Figure 3. Comparative γH2AX foci numbers present after low-dose chronic irradiation within populations differentially expressing ATM.



Source: Goodarzi AA, Jeggo P. Irradiation induced foci (IRIF) as a biomarker for radiosensitivity. Mutat Res. 2012 Aug 1;736(1-2):39-47.

Figure 4. Linear relationship of applied radiation dose and γH2AX intensity as measured by flow cytometry.

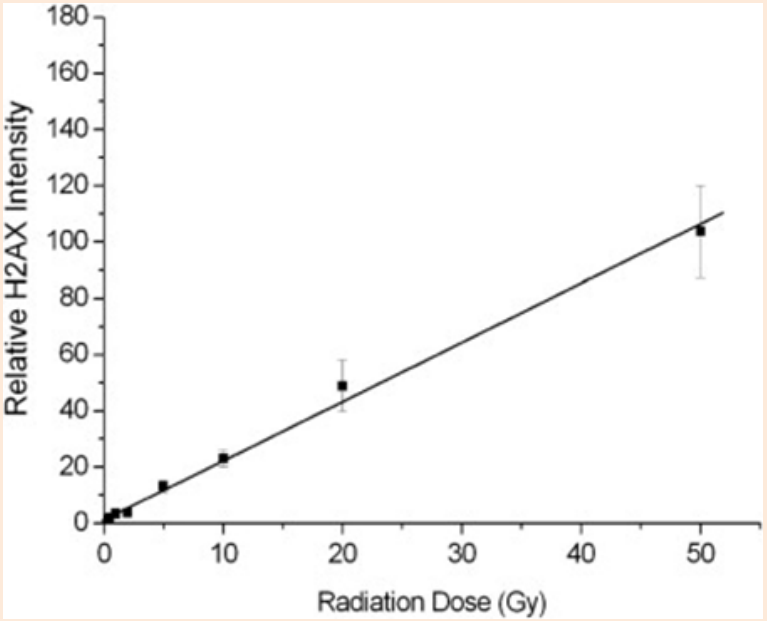
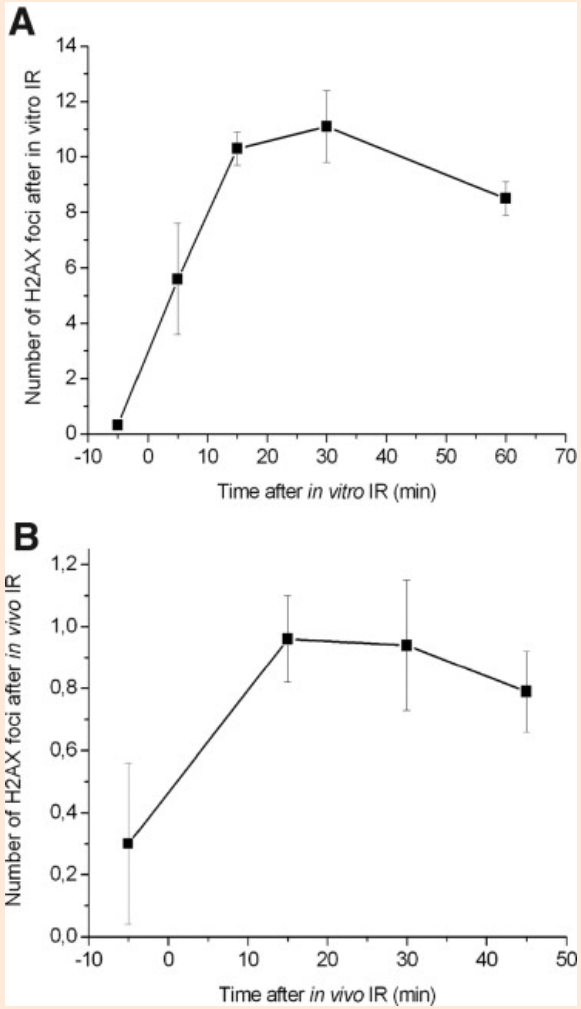


Figure 5. Comparative γH2AX foci numbers in lymphocytes before and after *in vivo* and *in vitro* irradiation.



Source: Sak A, Stuschke M. Use of γH2AX and other biomarkers of double-strand breaks during radiotherapy. Semin Radiat Oncol. 2010 Oct;20(4):223-31.

6. REFERENCES

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(2) *Uziel T, Lerenthal Y, Moyal L et al. Requirement of the MRN complex for ATM activation by DNA damage. EMBO J. 2003 Oct 15;22(20):5612-21. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC213795/*

(3) *Goodarzi AA, Jeggo P. Irradiation induced foci (IRIF) as a biomarker for radiosensitivity. Mutat Res. 2012 Aug 1;736(1-2):39-47. Available from: http://www.sciencedirect.com/science/article/pii/S0027510711001412*

(4) *Sak A, Stuschke M. Use of γH2AX and other biomarkers of double-strand breaks during radiotherapy. Semin Radiat Oncol. 2010 Oct;20(4):223-31. Available from: http://www.sciencedirect.com/science/article/pii/S105342961000038X*

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