



*DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY  
UNIVERSITY OF CANTABRIA, SANTANDER, SPAIN*

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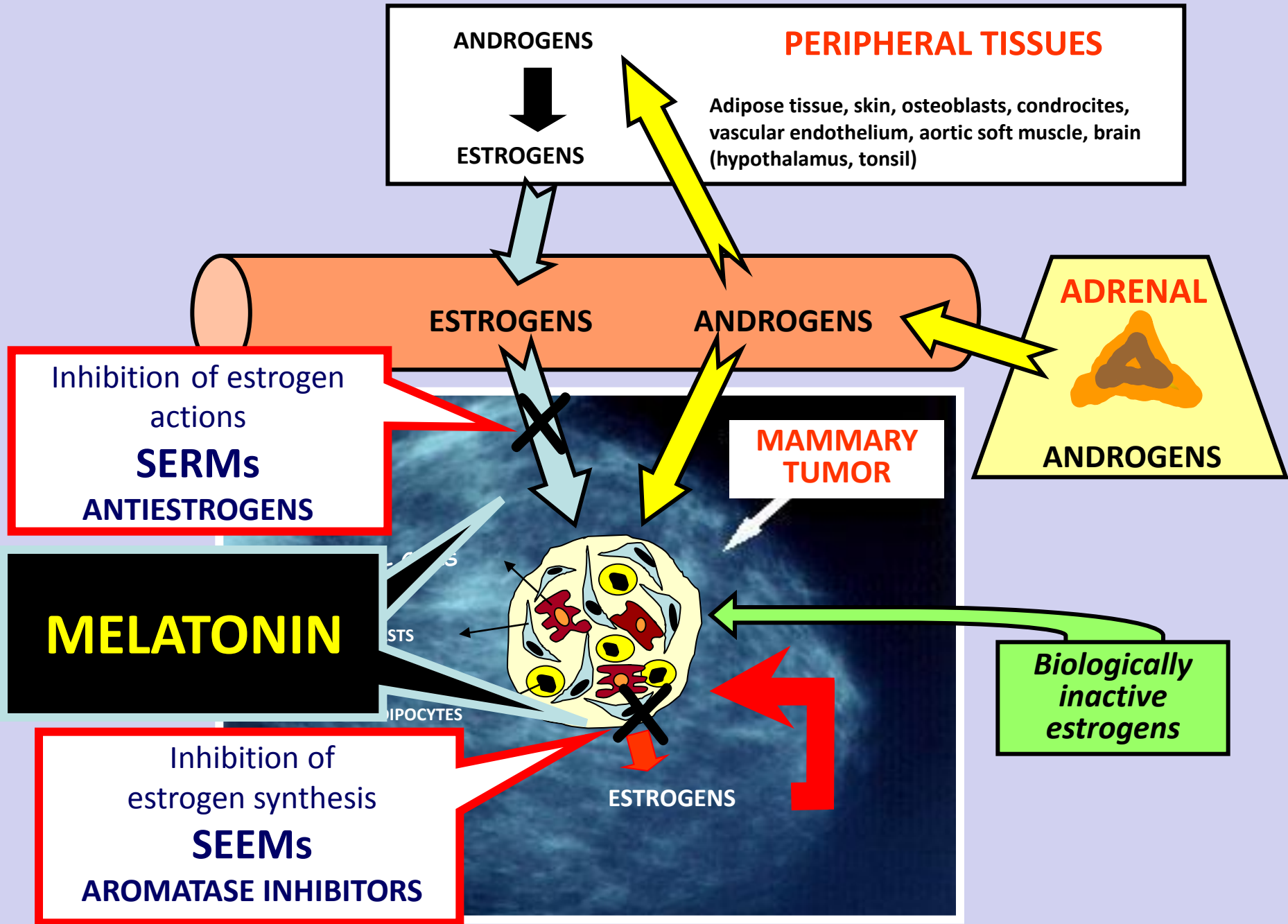
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# **MELATONIN SENSITIZES HUMAN BREAST CANCER CELLS TO IONIZING RADIATION**

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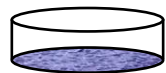
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*Carolina Alonso-González*

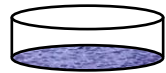


# **IRRADIATION**

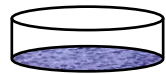
*(dose ranging 4-12 Gy)*



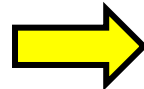
**Melatonin 1 mM**



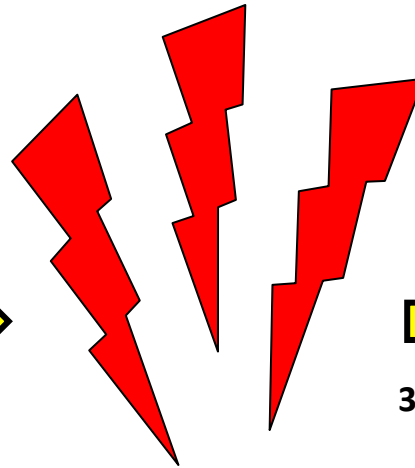
**Melatonin 10 μM**



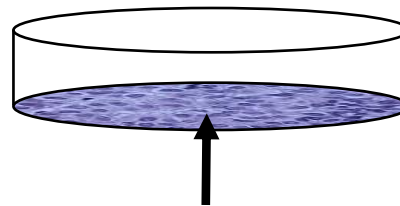
**Melatonin 1 nM**



**7 Days**



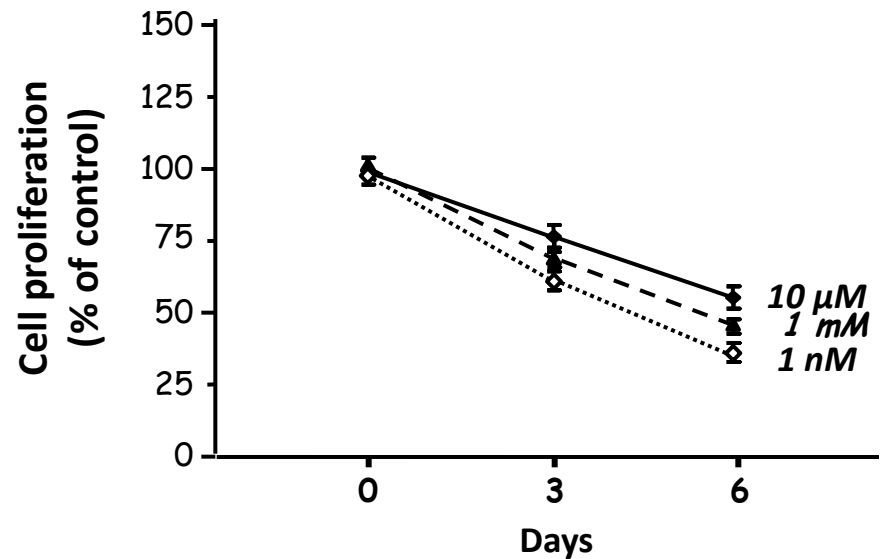
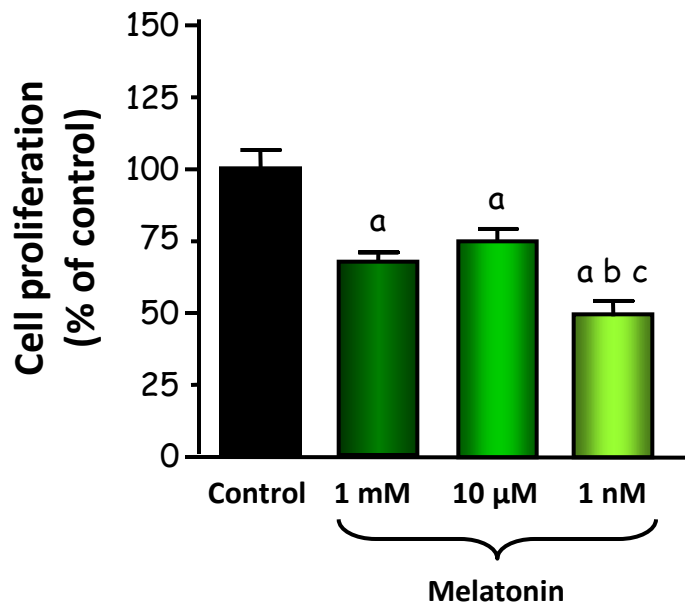
**3-6 days**



**Malignant epithelial cells  
MCF-7**

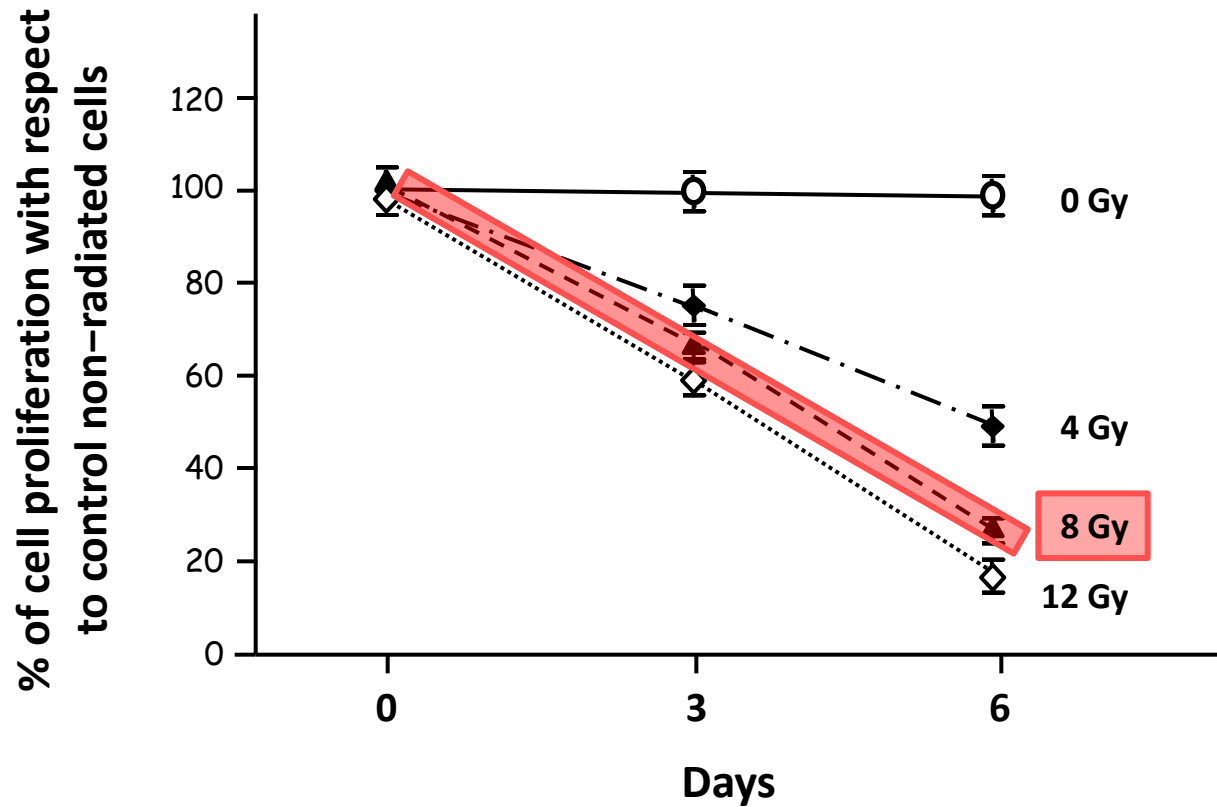
- **Cell proliferation**
- **Cell cycle phase distribution**
- **RAD51 and DNA-PKs gene expression**

# EFFECTS OF MELATONIN ON BREAST CANCER CELL PROLIFERATION

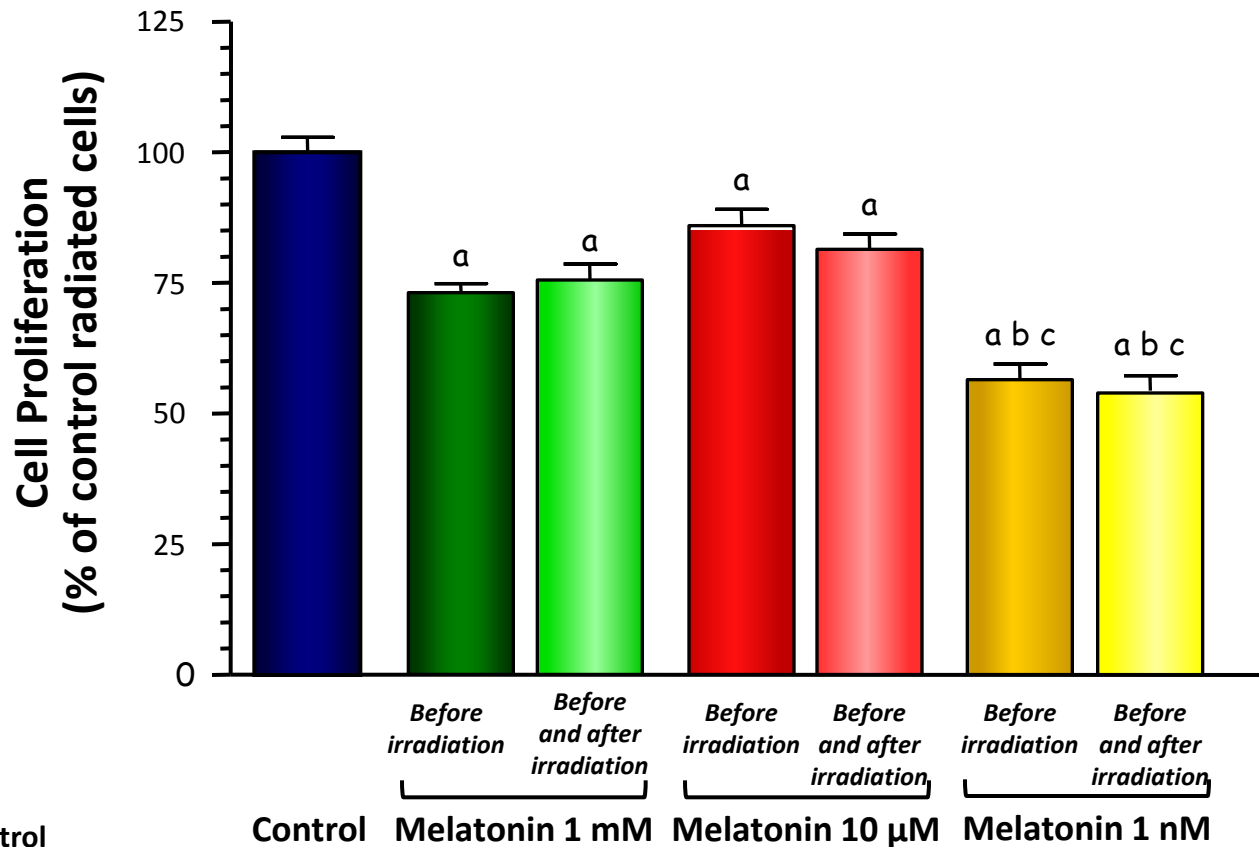


a,  $p < 0.001$  vs Control  
b,  $p < 0.001$  vs Melatonin 1 mM  
c,  $p < 0.01$  vs Melatonin 10 μM

# EFFECTS OF IONIZING RADIATION ON BREAST CANCER CELL PROLIFERATION (MCF-7)

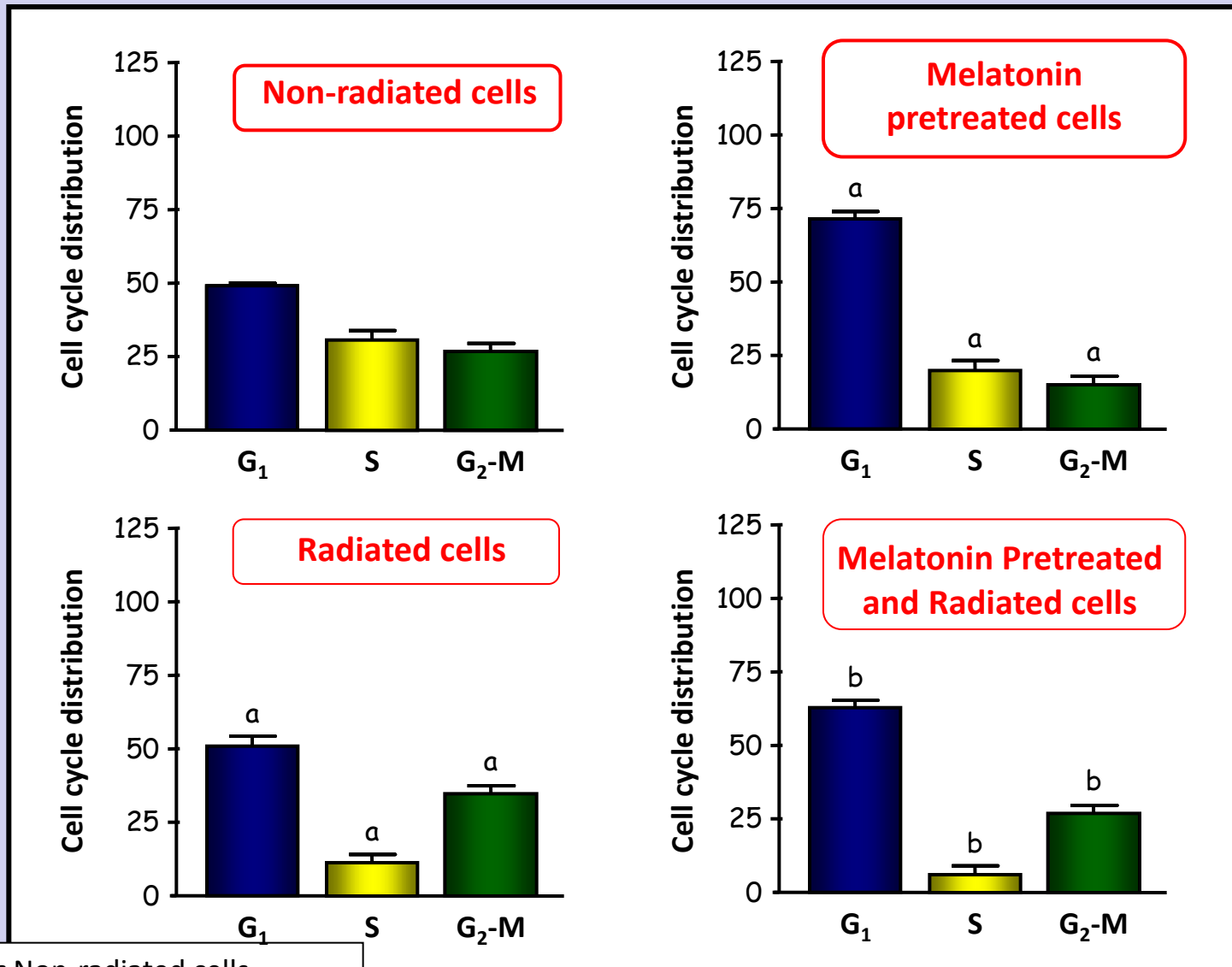


# POTENTIATION OF RADIATION-INDUCED GROWTH INHIBITION BY MELATONIN



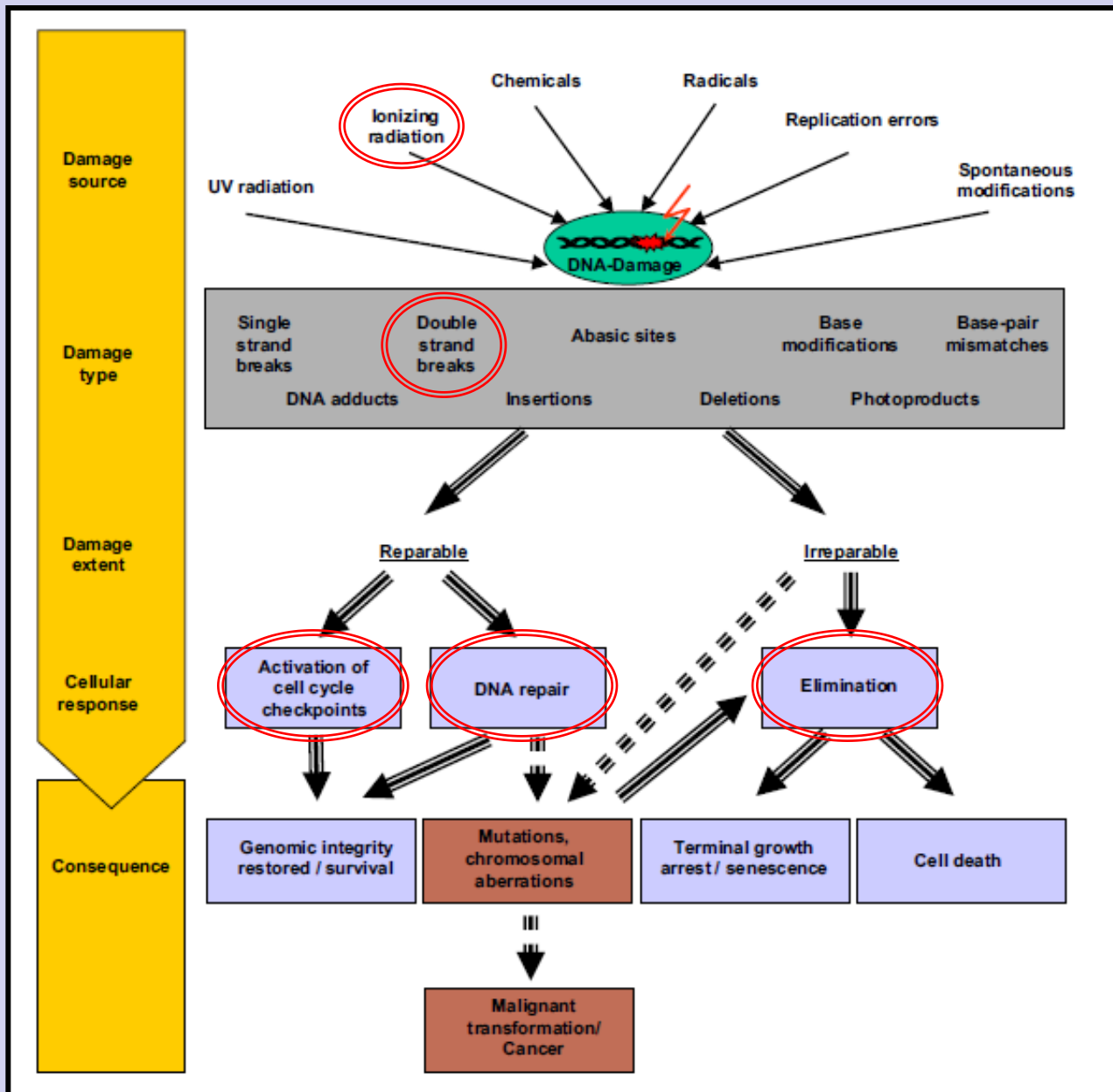
a,  $p < 0.001$  vs Control  
b,  $p < 0.001$  vs Melatonin 1 mM  
c,  $p < 0.01$  vs Melatonin 10  $\mu$ M

# CELL CYCLE DISTRIBUTION IN MCF-7 CELLS AFTER TREATMENT WITH MELATONIN AND IONIZING RADIATION

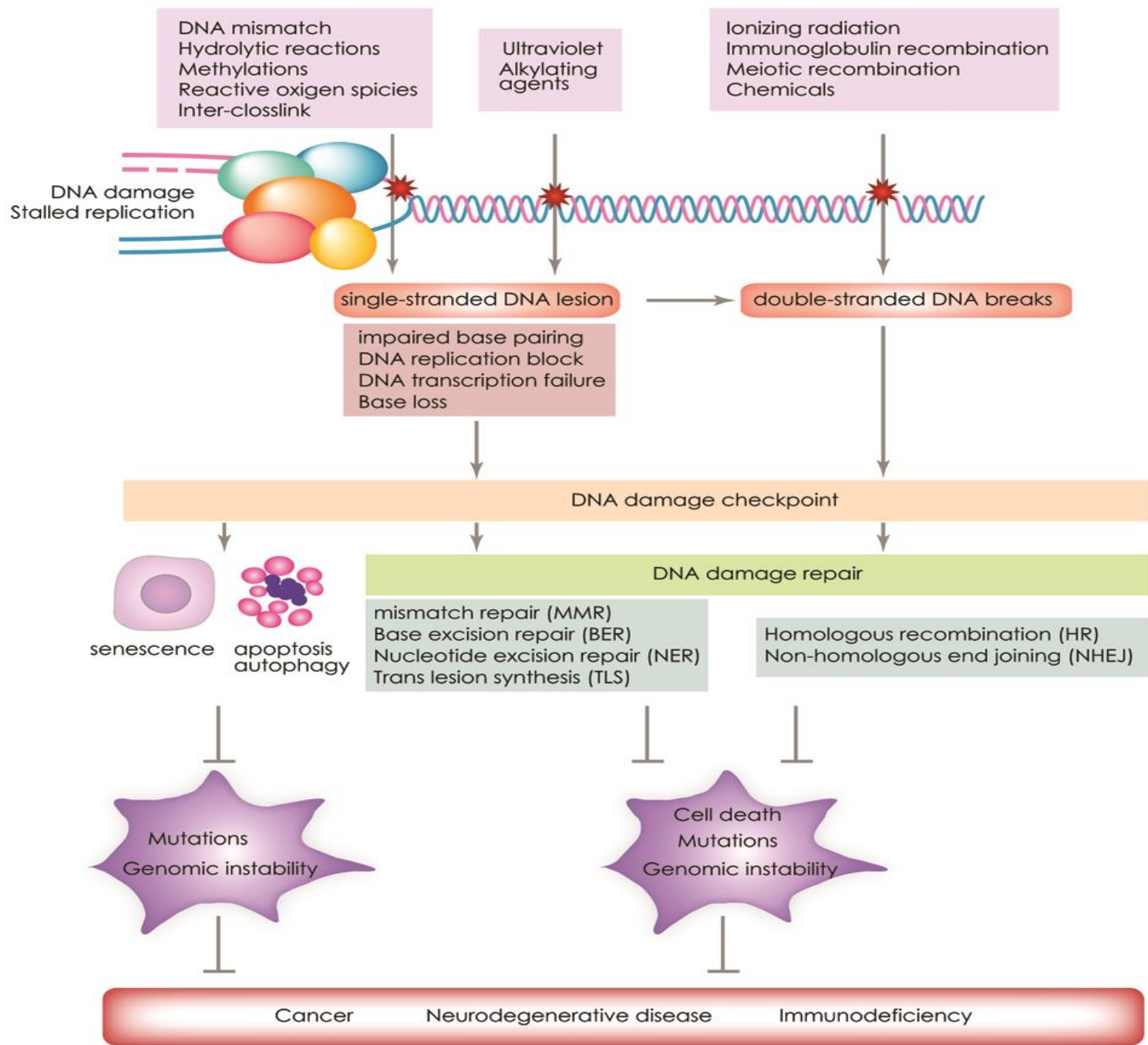


a, p<0.001 vs Non-radiated cells

b, p<0.001 vs Radiated cells









**DOUBLE STRAND BREAKS (DSBs)**

**ATM  
MRN COMPLEX**

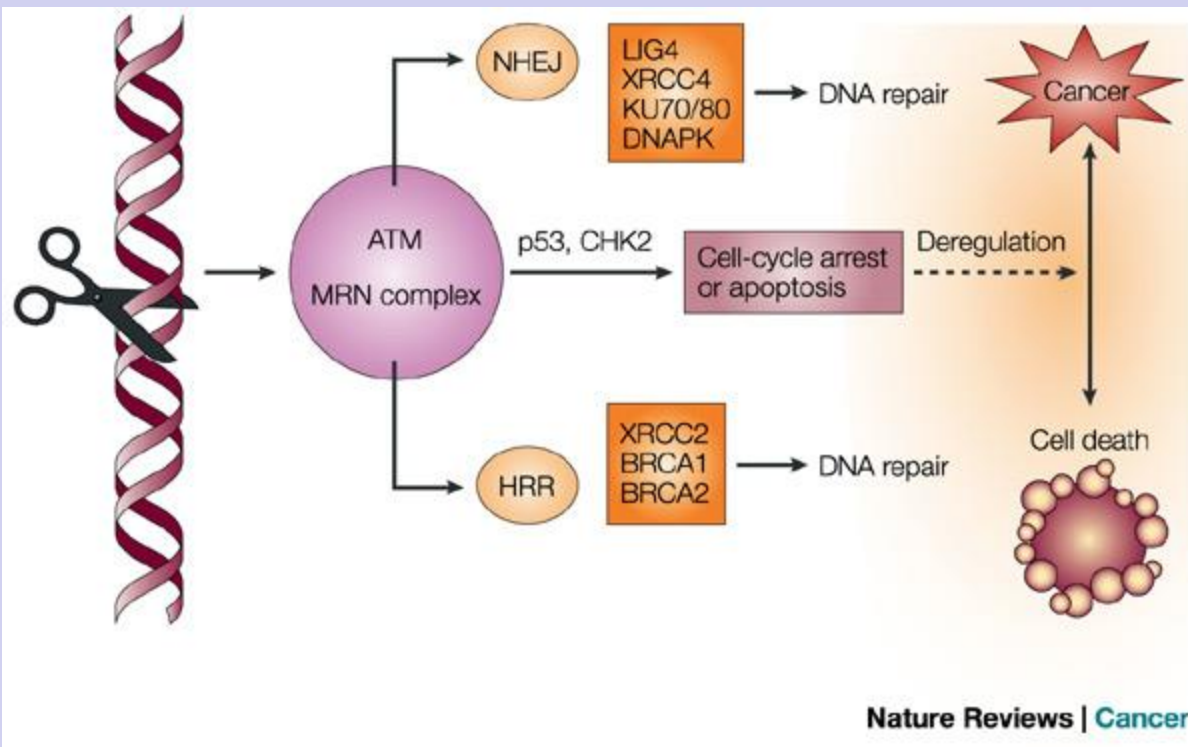
**P53  
CHK2**

- Apoptosis
- Senescence

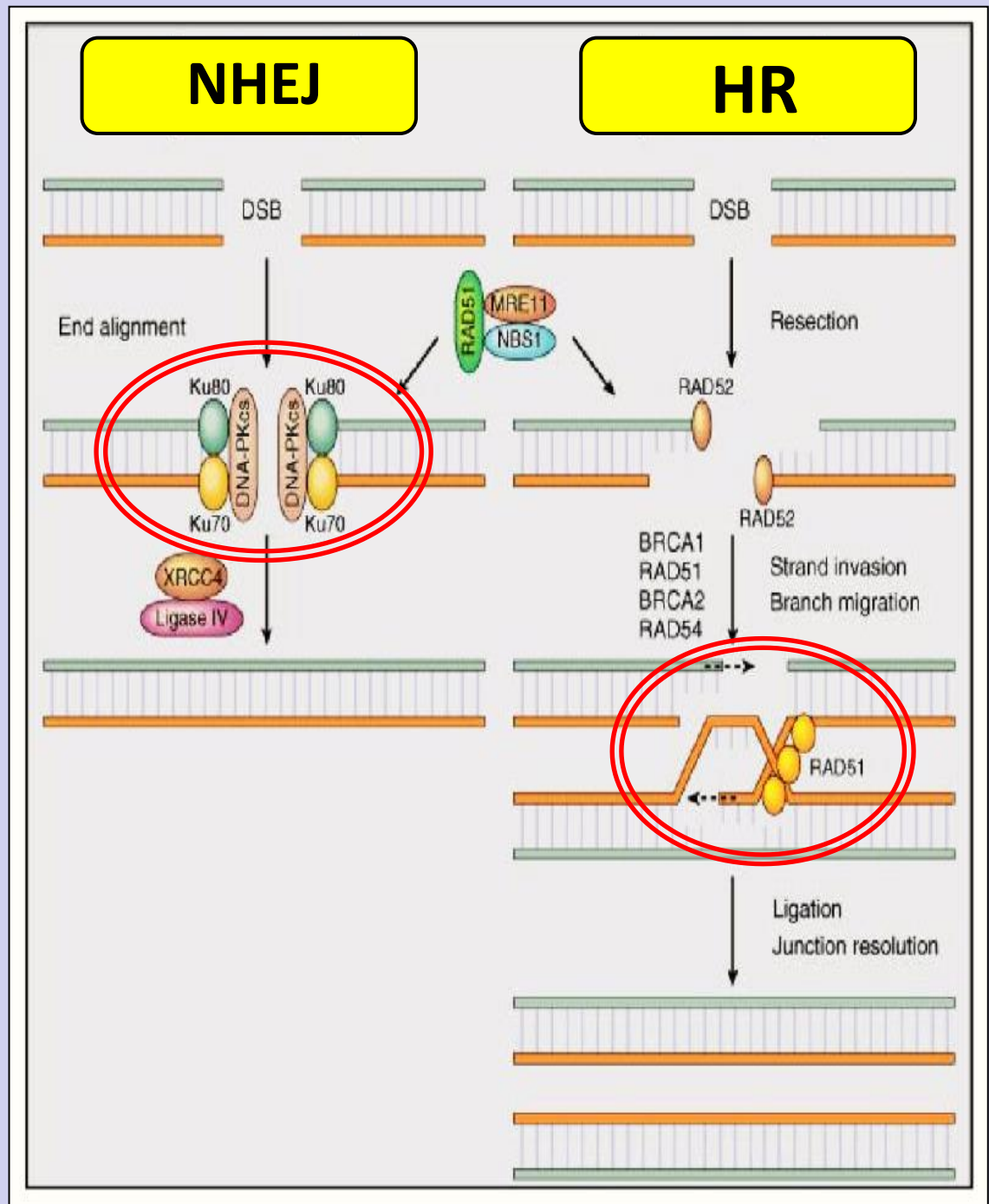
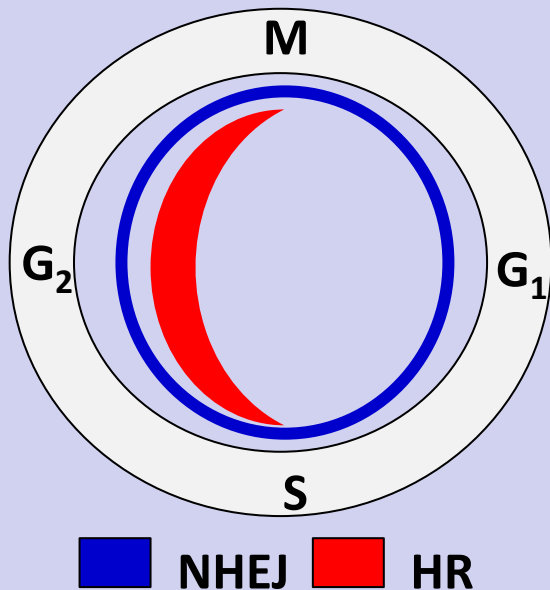
**P53  
CHK2**

- Cell cycle arrest
- DNA damage repair

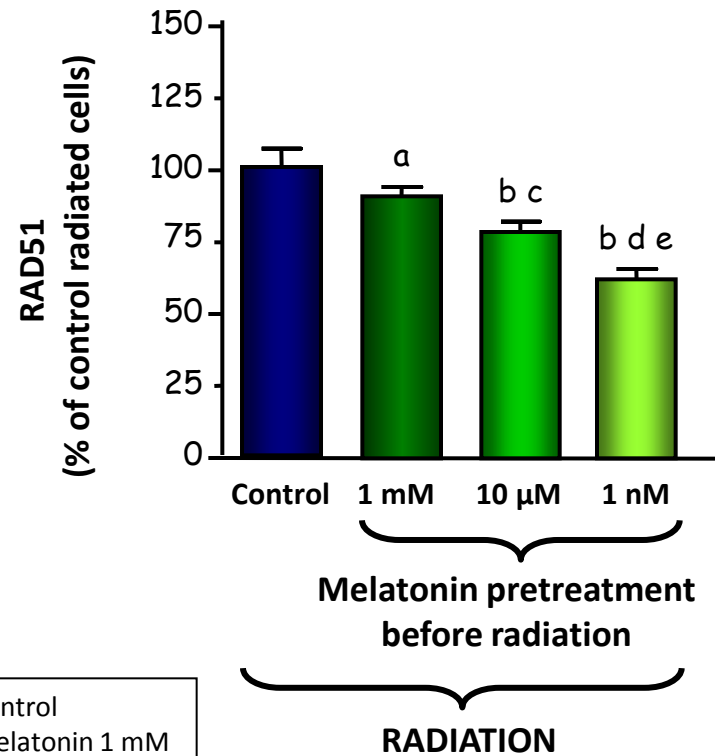
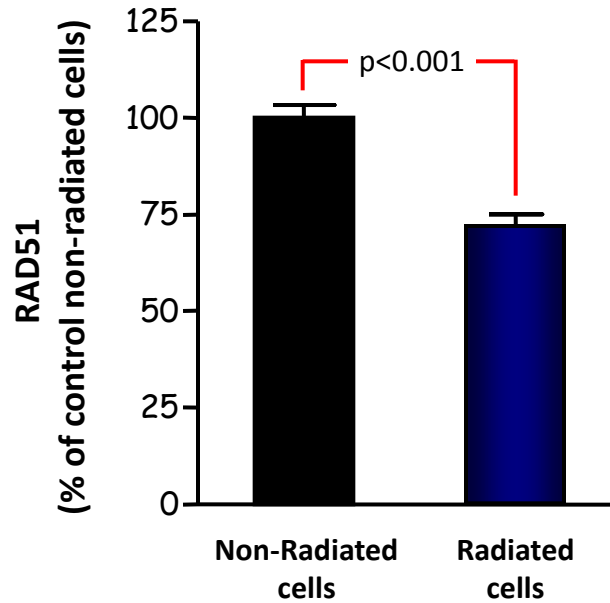
**Genomic Integrity Restored  
SURVIVAL**



# REPAIR OF DNA DOUBLE STRAND BREAKS (DSBs)

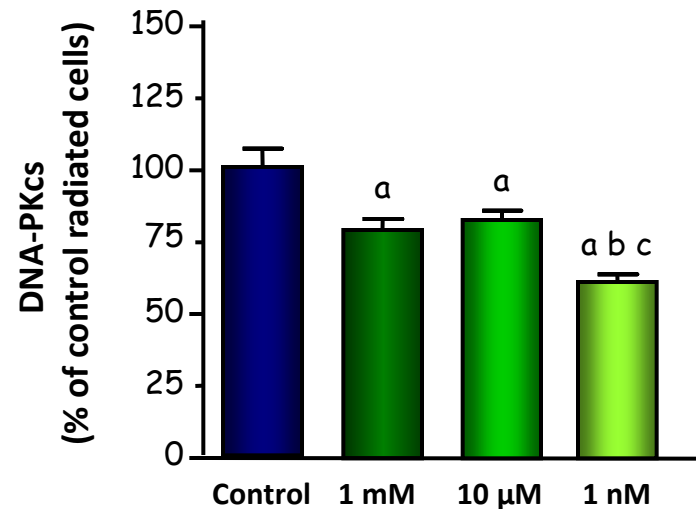
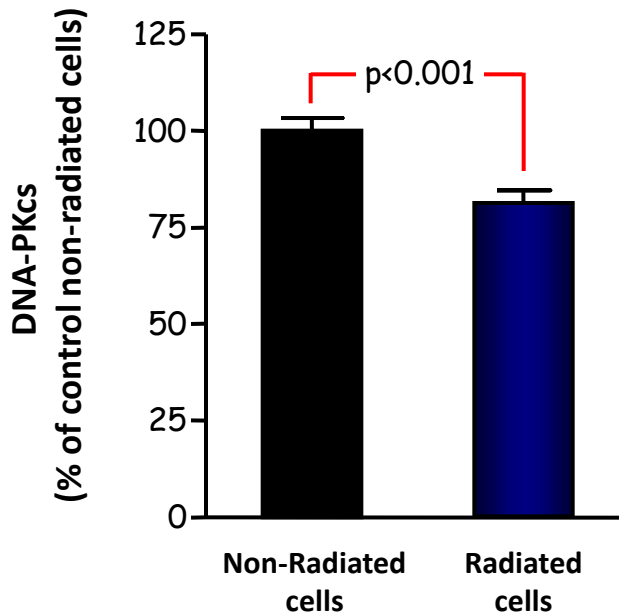


# EFFECTS OF RADIATION AND MELATONIN ON RAD51 mRNA EXPRESSION IN MCF-7 CELLS

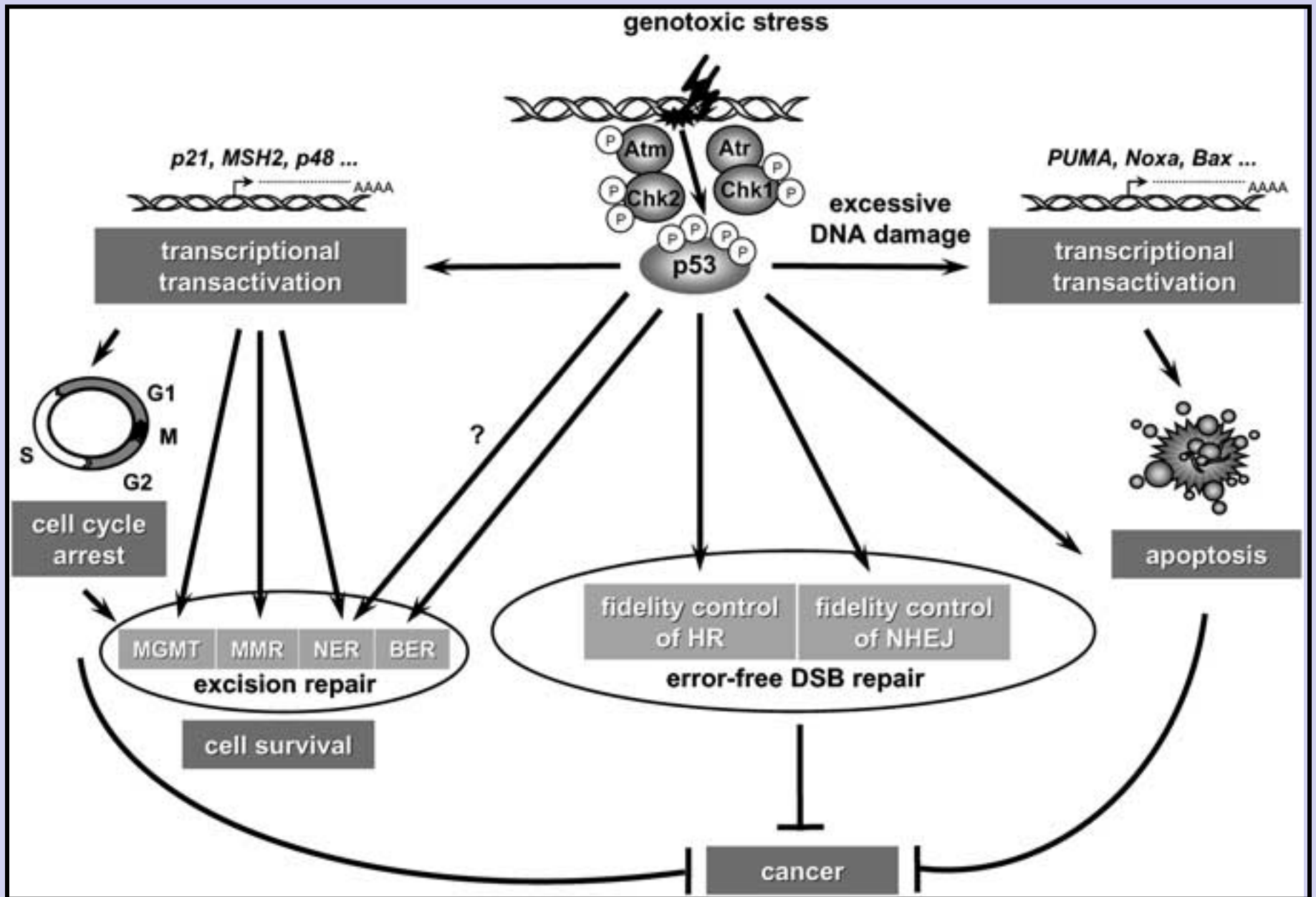


a, p<0.001 vs Control  
b, p<0.001 vs Melatonin 1 mM  
c, p<0.001 vs Melatonin 10 μM

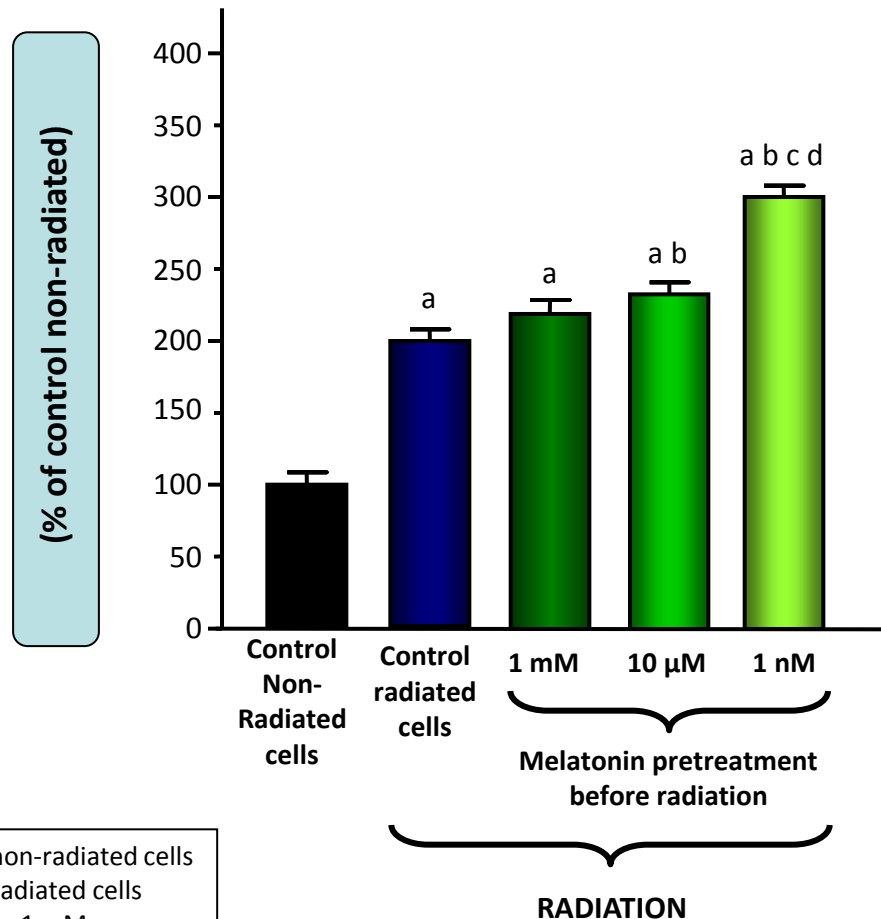
# EFFECTS OF RADIATION AND MELATONIN ON DNA-PKcs mRNA EXPRESSION IN MCF-7 CELLS



a,  $p < 0.01$  vs Control  
 b,  $p < 0.001$  vs Control  
 c,  $p < 0.01$  vs Melatonin 1 mM  
 d,  $p < 0.001$  vs Melatonin 1 mM  
 e,  $p < 0.01$  vs Melatonin 10 μM



# EFFECTS OF IONIZING RADIATION AND MELATONIN ON p53 mRNA EXPRESSION IN BREAST CANCER CELLS



a,  $p < 0.001$  vs Control non-irradiated cells  
b,  $p < 0.001$  vs Control radiated cells  
c,  $p < 0.001$  vs Melatonin 1 mM  
d,  $p < 0.001$  vs Melatonin 10 μM



**MELATONIN  
PRETREATMENT  
BEFORE RADIATION  
SENSITIZES BREAST  
CANCER CELLS TO  
THE IONIZING  
EFFECTS OF  
RADIATION**



**Decreasing cell  
proliferation.**

**Inducing cell cycle  
arrest.**

**Down-regulating  
RAD-51 and also  
DNA-PKc.**  
Two proteins  
involved in double-  
strand DNA break  
repair mechanisms.



# RESEARCH GROUP

**CAROLINA ALONSO-GONZÁLEZ**

**ALICIA GONZÁLEZ CABEZA**

**CARLOS MARTÍNEZ CAMPA**

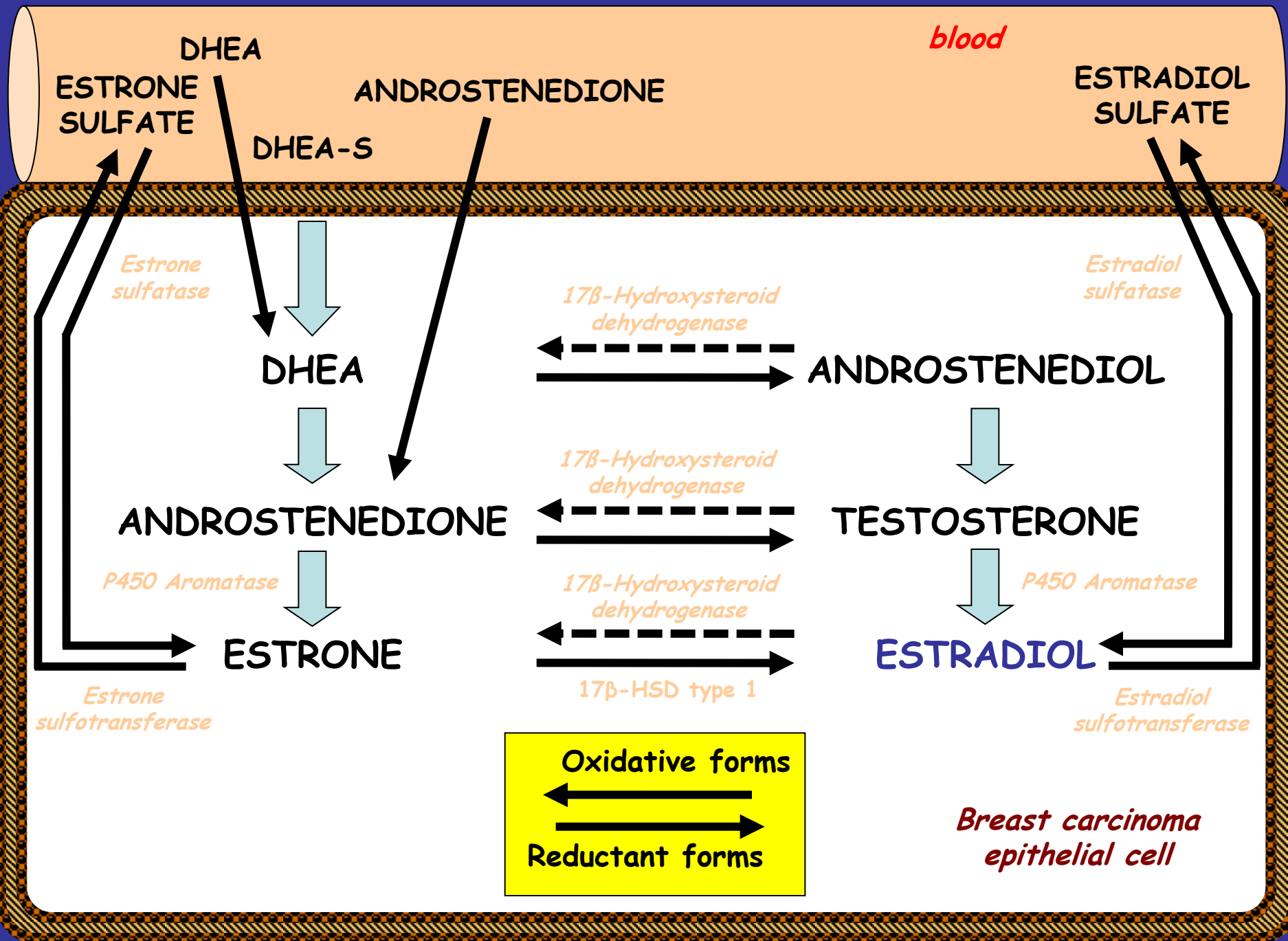
**JAVIER MENÉNDEZ MENÉNDEZ**

**SAMUEL COS CORRAL**

**Technical assistance**

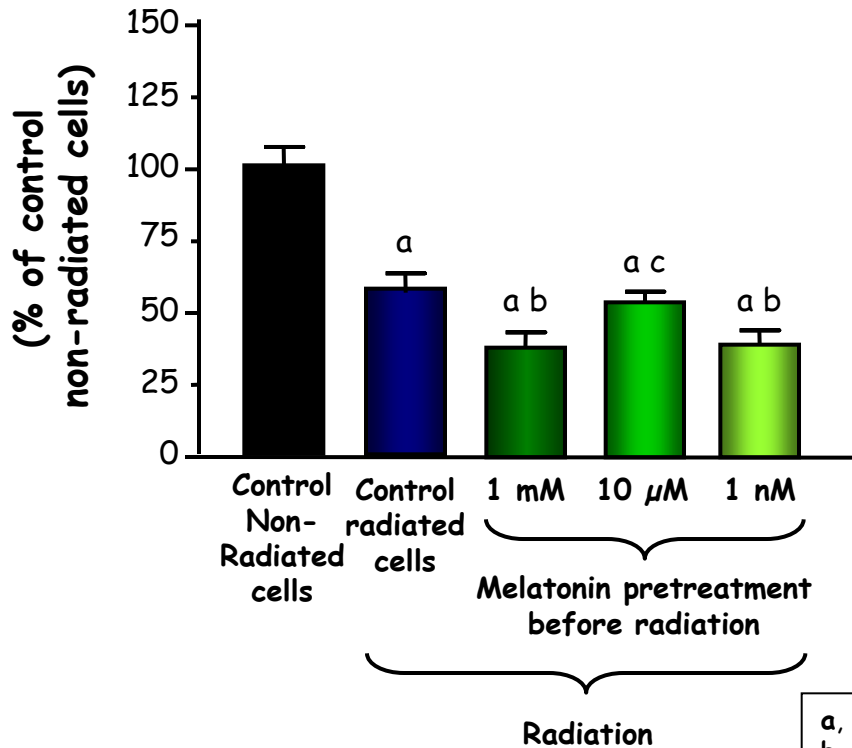
**José Antonio de Cos Cossío**

**Gema Viar Ruíz**

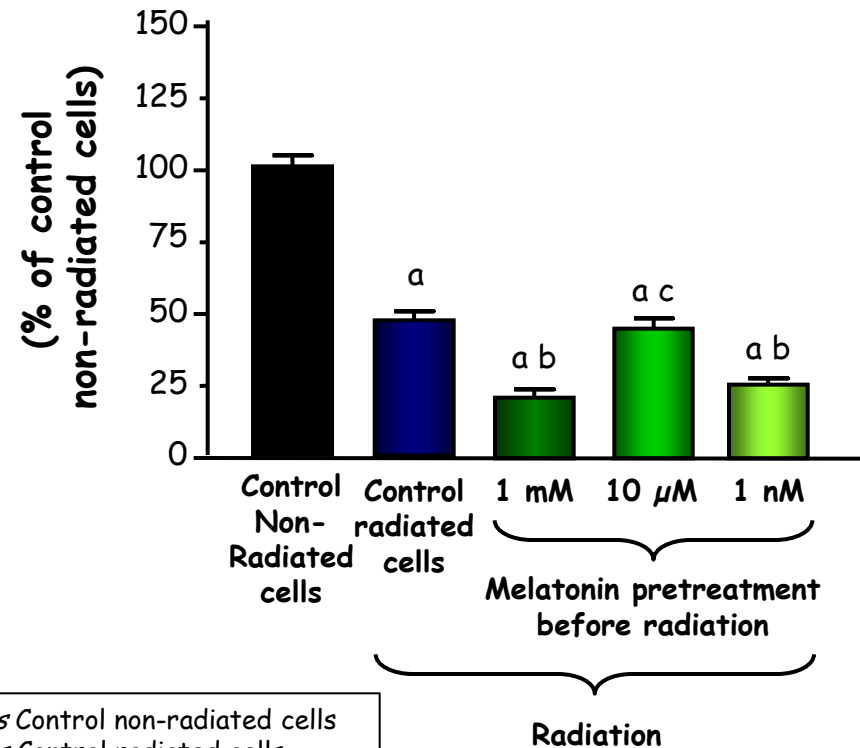


# EFFECTS OF IONIZING RADIATION AND MELATONIN ON AROMATASE ACTIVITY AND mRNA EXPRESSION IN HUMAN BREAST CANCER CELLS

## AROMATASE ACTIVITY



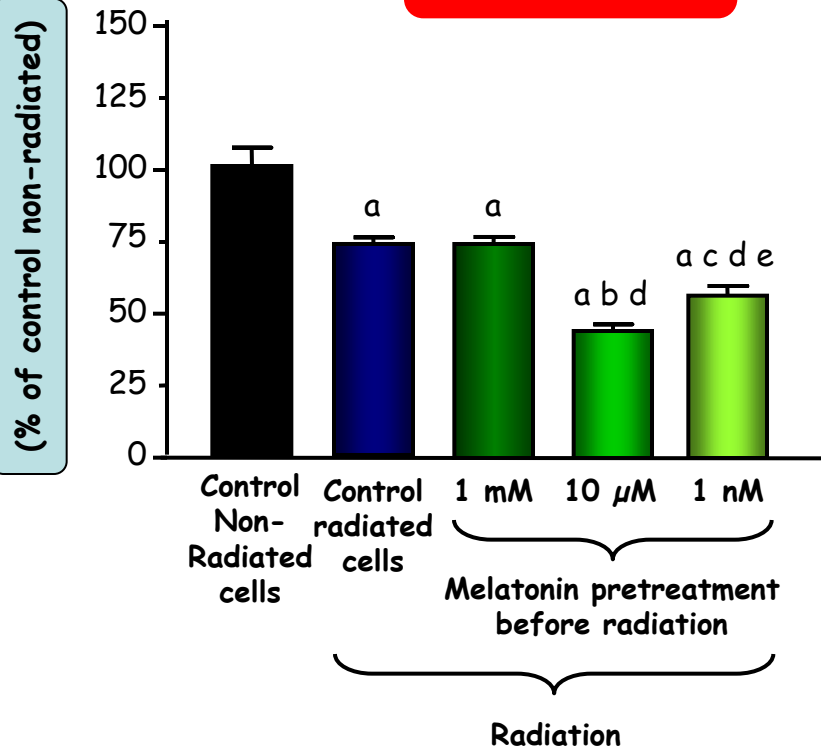
## AROMATASE mRNA EXPRESSION



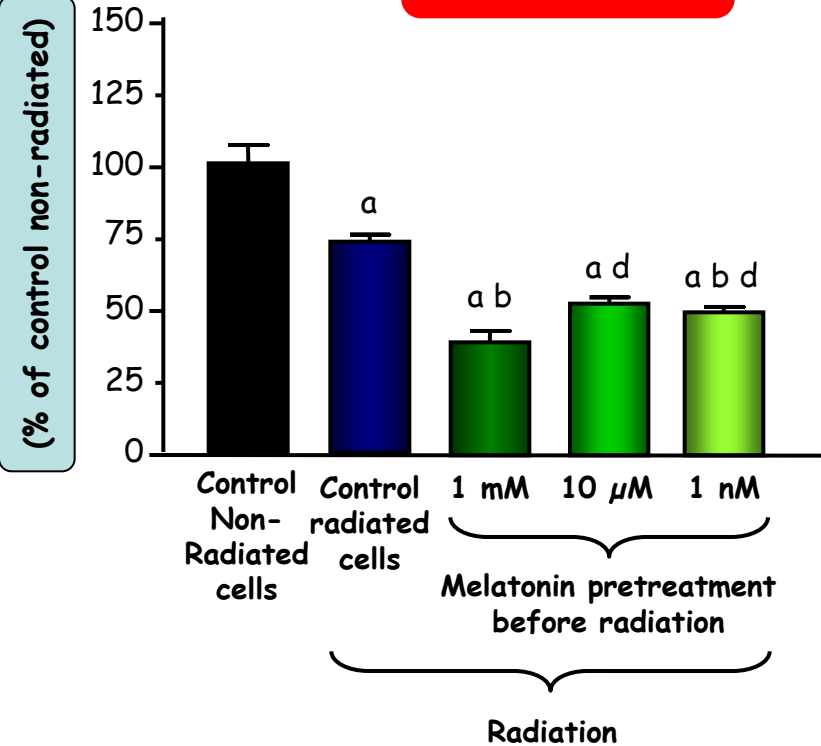
a,  $p < 0.001$  vs Control non-radiated cells  
 b,  $p < 0.001$  vs Control radiated cells  
 c,  $p < 0.001$  vs Melatonin 1 mM

# EFFECTS OF IONIZING RADIATION AND MELATONIN ON THE TWO MAJOR PROMOTERS (pI.3 AND pII) DRIVING AROMATASE EXPRESSION IN BREAST CANCER

Promoter I.3

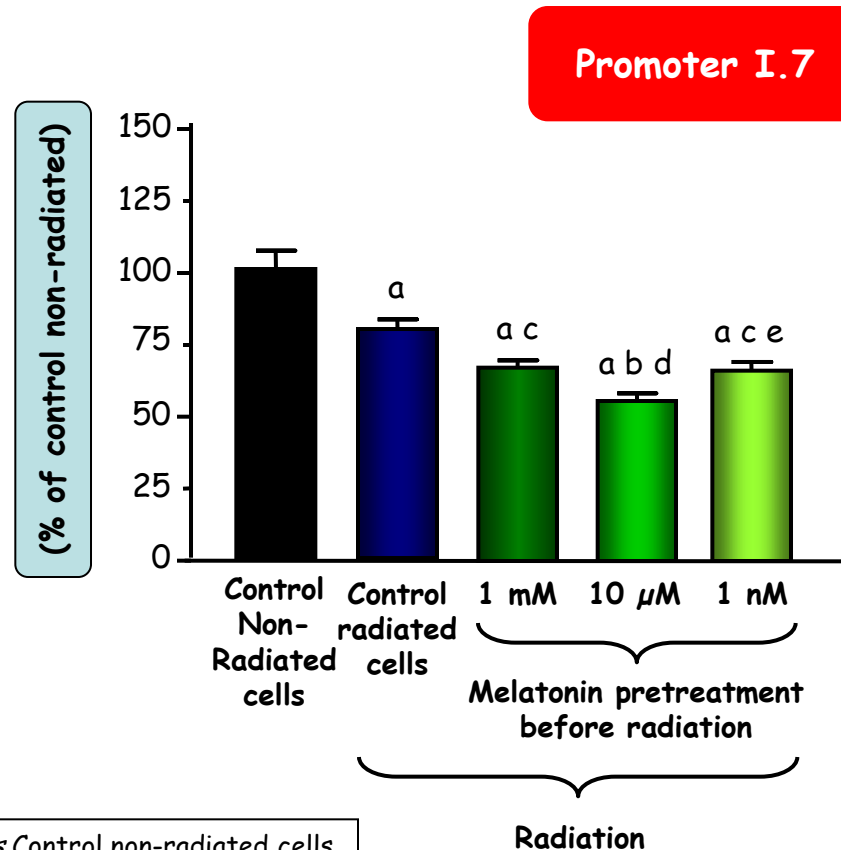


Promoter II



a,  $p < 0.001$  vs Control non-radiated cells  
 b,  $p < 0.001$  vs Control radiated cells  
 c,  $p < 0.01$  vs Control radiated cells  
 d,  $p < 0.001$  vs Melatonin 1 mM  
 e,  $p < 0.001$  vs Melatonin 10 μM

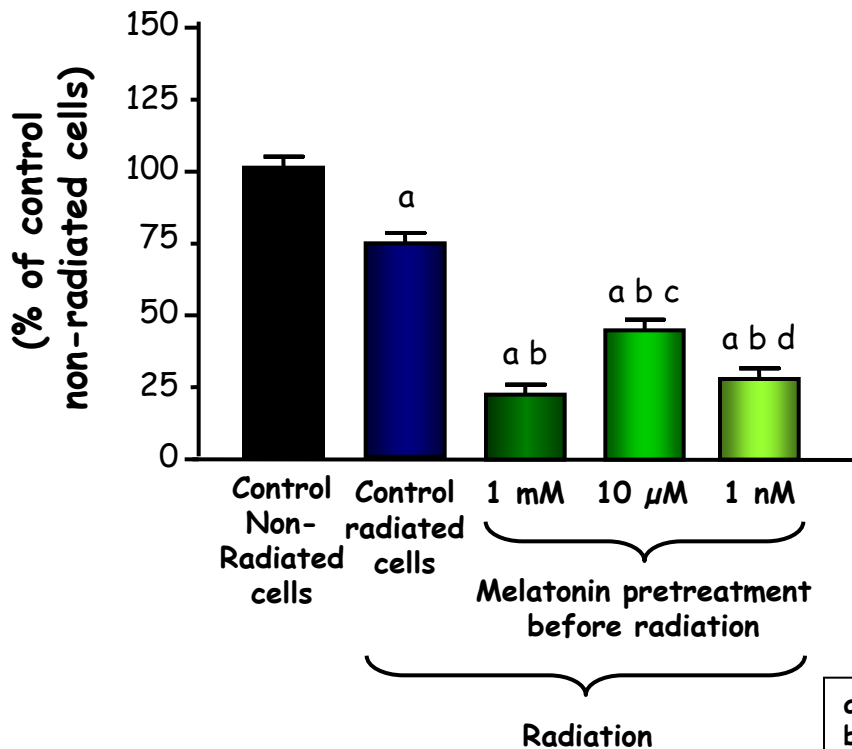
# EFFECTS OF IONIZING RADIATION AND MELATONIN ON AROMATASE PROMOTER I.7 mRNA EXPRESSION IN BREAST CANCER CELLS



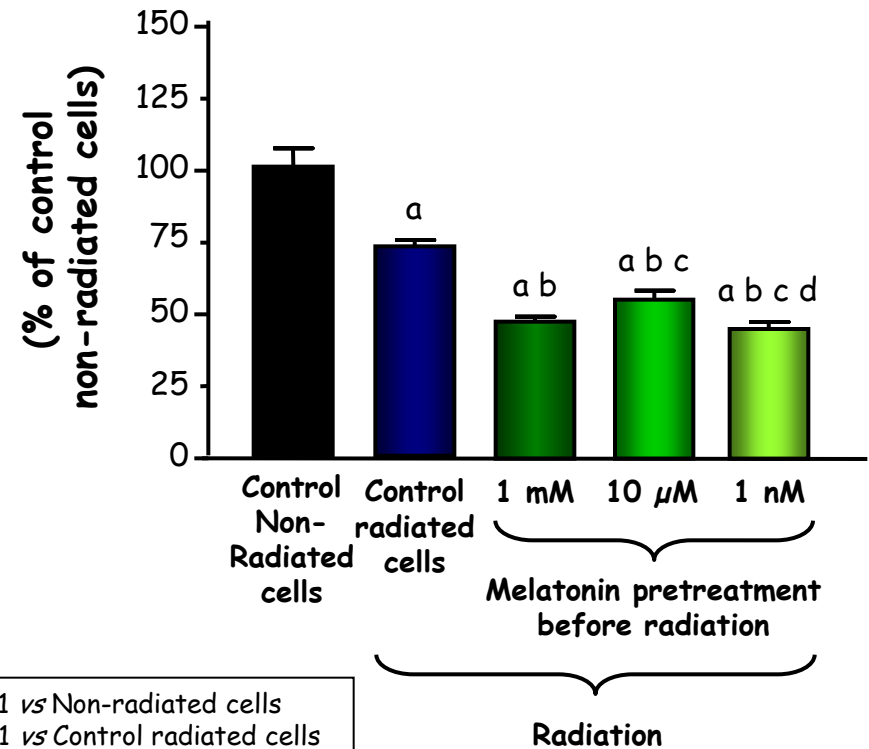
- a,  $p < 0.001$  vs Control non-radiated cells
- b,  $p < 0.001$  vs Control radiated cells
- c,  $p < 0.01$  vs Control radiated cells
- d,  $p < 0.01$  vs Melatonin 1 mM
- e,  $p < 0.01$  vs Melatonin 10  $\mu\text{M}$

# EFFECTS OF IONIZING RADIATION AND MELATONIN ON SULFATASE ACTIVITY AND mRNA EXPRESSION IN HUMAN BREAST CANCER CELLS

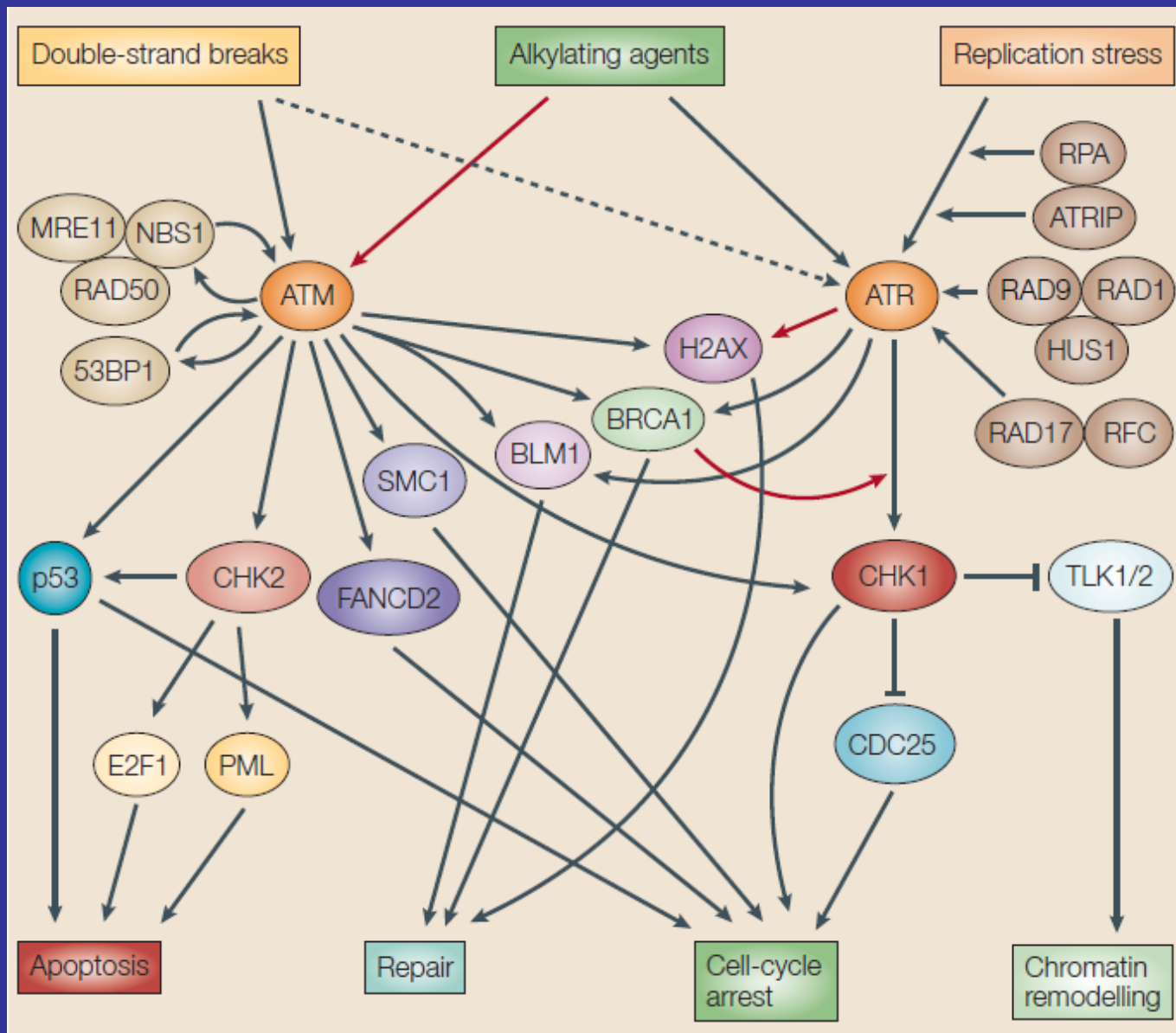
## SULFATASE ACTIVITY



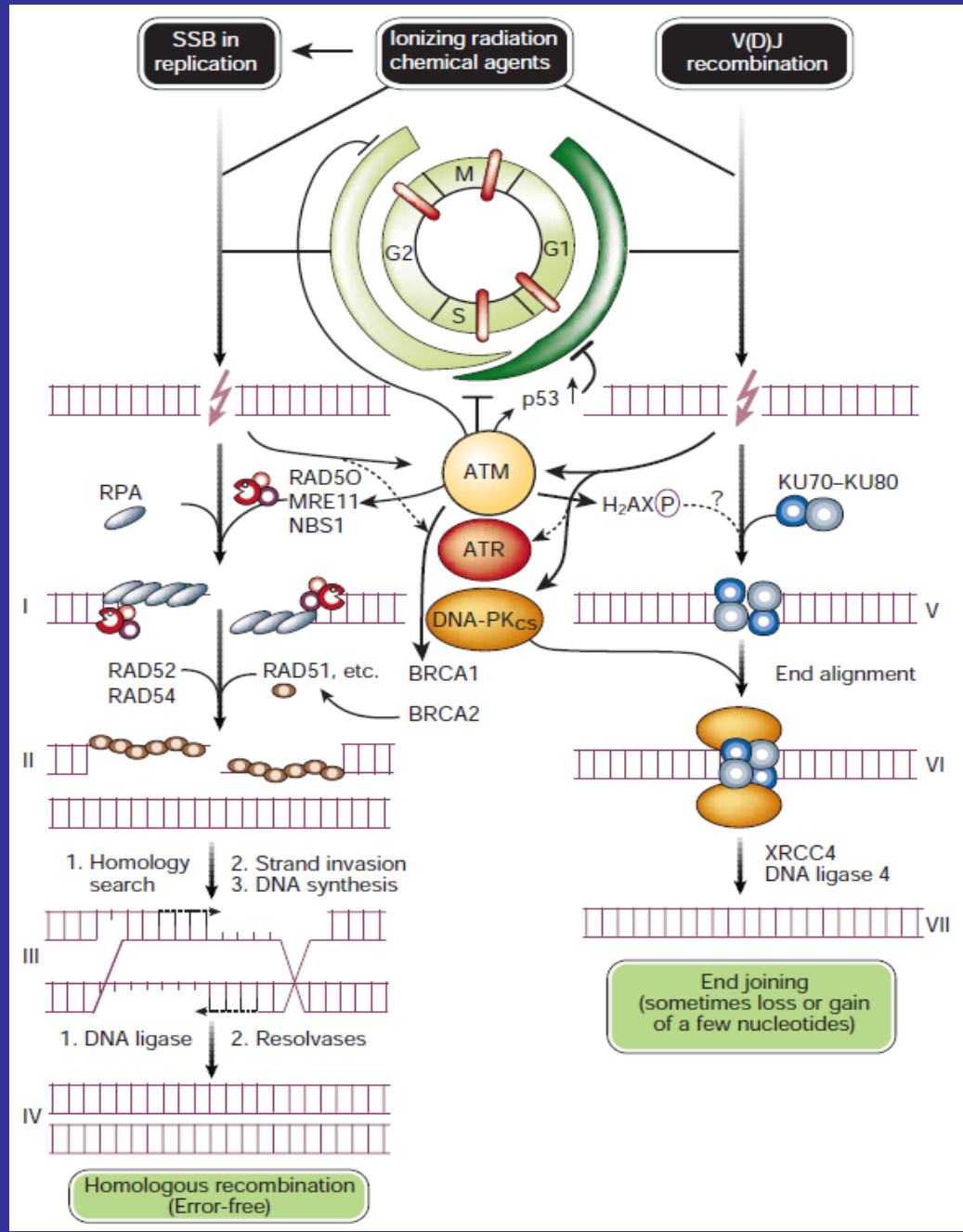
## SULFATASE mRNA EXPRESSION

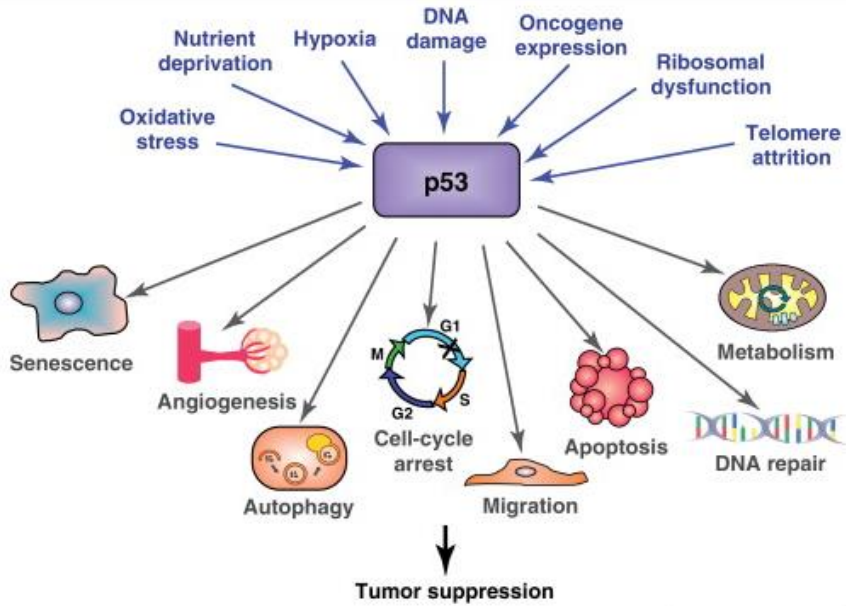


a,  $p < 0.001$  vs Non-radiated cells  
 b,  $p < 0.001$  vs Control radiated cells  
 c,  $p < 0.01$  vs Melatonin 1 mM  
 d,  $p < 0.001$  vs Melatonin 10 μM



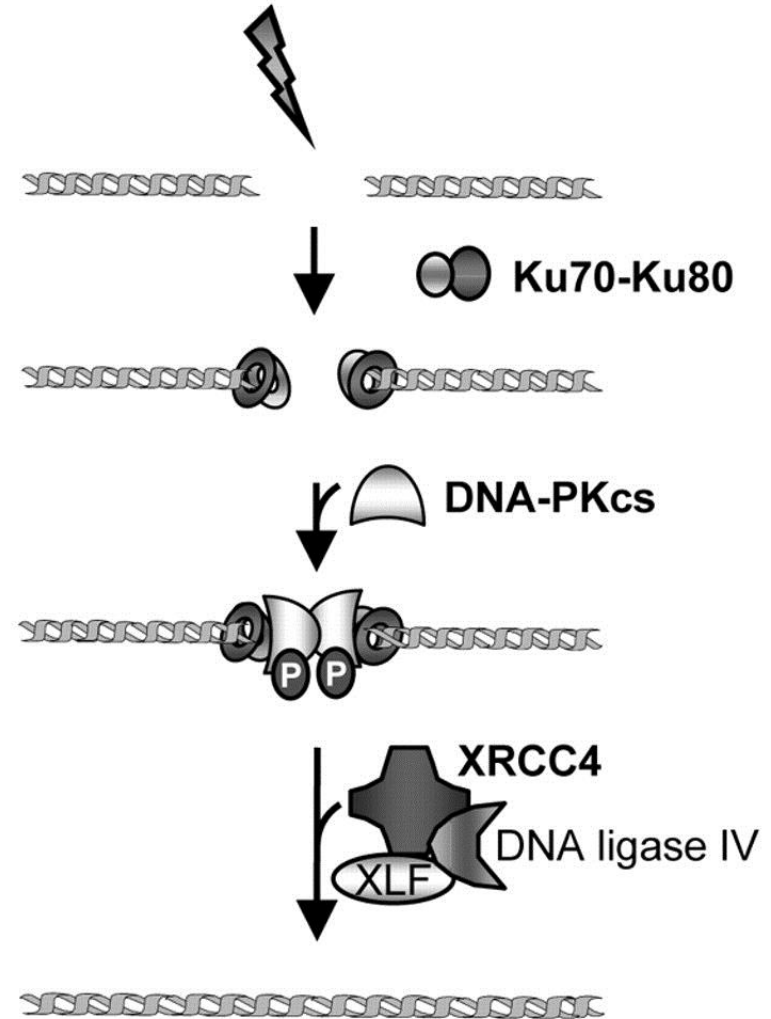






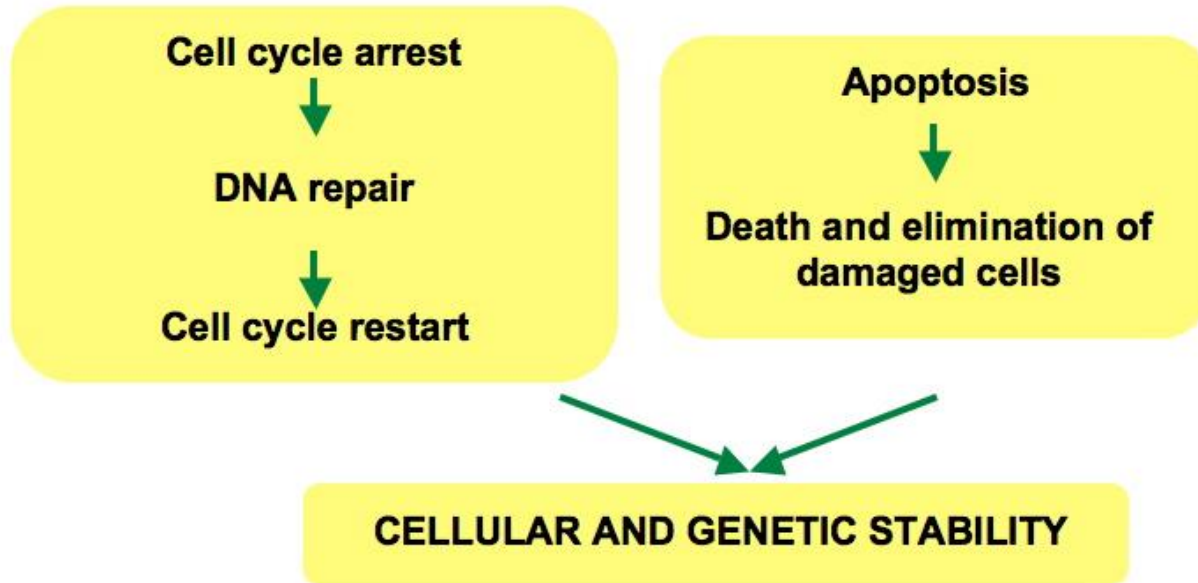
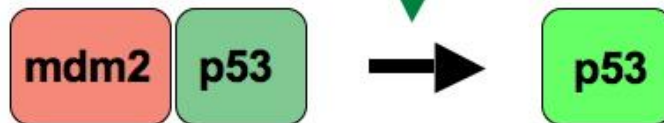
*TRENDS in Cell Biology*

## Ionizing radiation Radiomimetics agents (CL $\gamma$ 1)



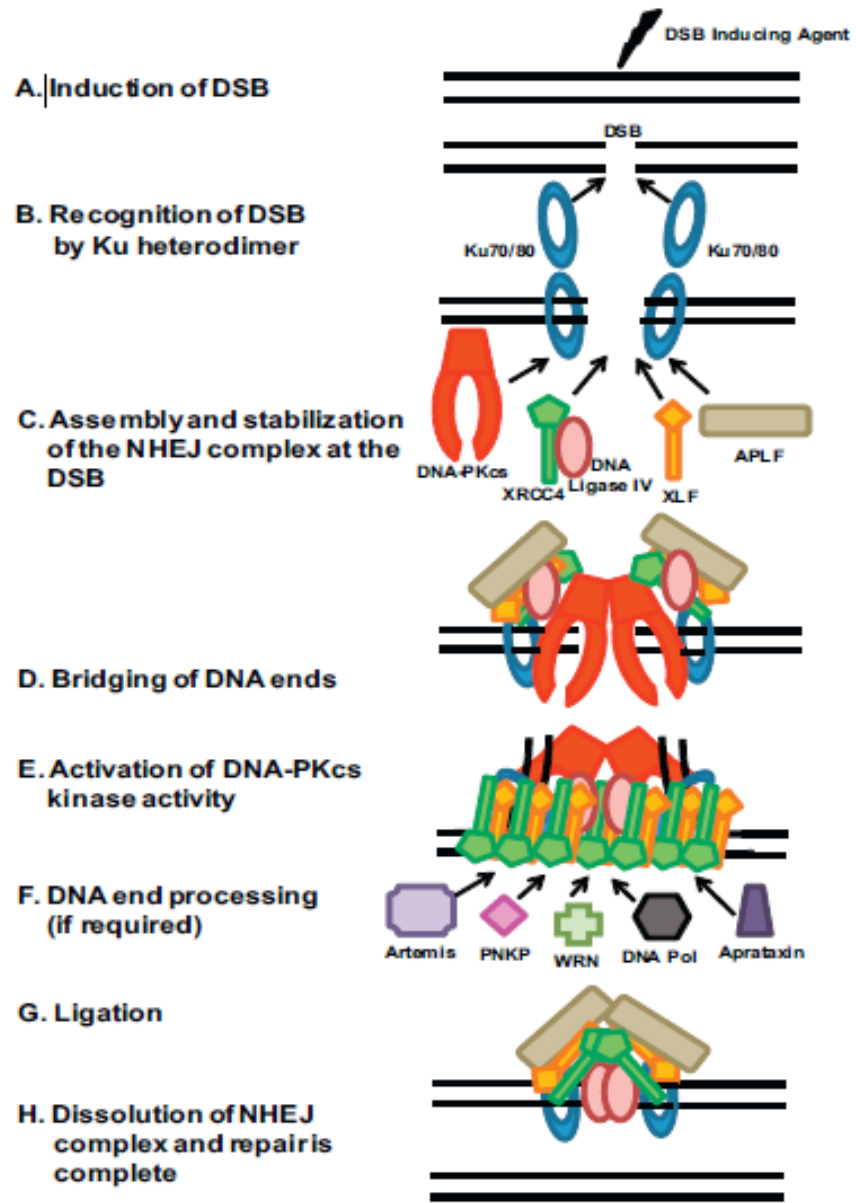
**Repair of DNA DSBs**

**DNA damage**  
**Cell cycle abnormalities**  
**Hypoxia**



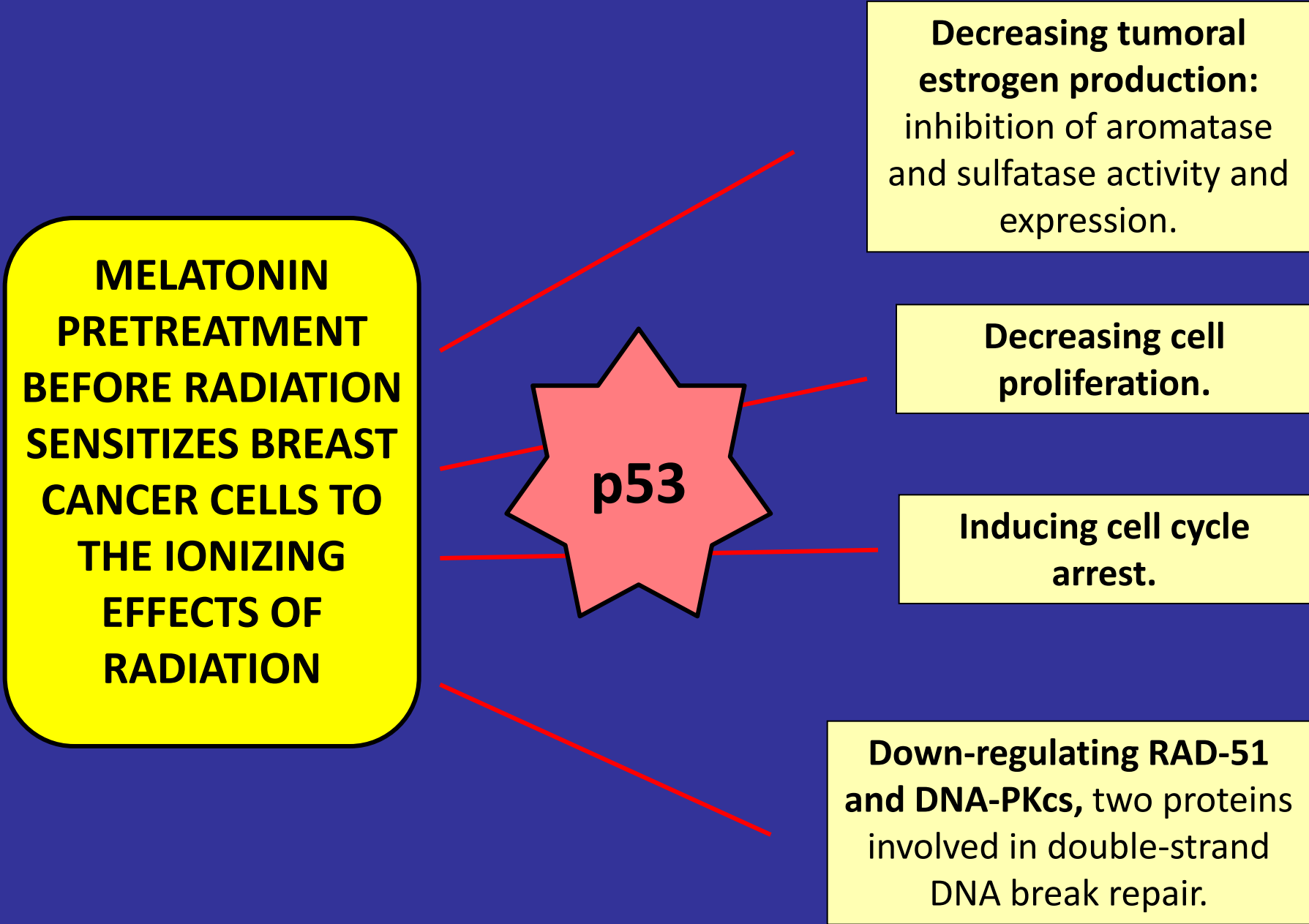
# CELL CYCLE DISTRIBUTION IN MCF-7 CELLS AFTER TREATMENT WITH MELATONIN AND IONIZING RADIATION

	Control	Melatonin		
		1 mM	10 $\mu$ M	1 nM
<i>0 h</i>				
$G_1$	51 $\pm$ 0.8	67 $\pm$ 0.6 *	65.3 $\pm$ 1.3 *	69 $\pm$ 0.3 *
S	28 $\pm$ 1.1	19 $\pm$ 1.2 *	19.9 $\pm$ 0.9 *	18 $\pm$ 0.8 *
$G_2/M$	21 $\pm$ 1.1	14 $\pm$ 0.8 *	14.3 $\pm$ 1.1 *	13 $\pm$ 0.2 *
<i>6 h after treatment</i>				
$G_1$	25.8 $\pm$ 0.7	42 $\pm$ 0.6 *	31.2 $\pm$ 0.6	31.9 $\pm$ 0.5
S	45.8 $\pm$ 0.5	38 $\pm$ 1.2	43.4 $\pm$ 0.5	42.2 $\pm$ 0.9
$G_2/M$	28.4 $\pm$ 0.6	20 $\pm$ 0.8 *	25.3 $\pm$ 1.2	25.8 $\pm$ 0.4
<i>12 h after treatment</i>				
$G_1$	58.4 $\pm$ 0.4	58.1 $\pm$ 0.6	52.3 $\pm$ 0.6	51.6 $\pm$ 0.5
S	7.1 $\pm$ 0.1	6.1 $\pm$ 1.2	6.3 $\pm$ 0.5	6.2 $\pm$ 0.9
$G_2/M$	33.6 $\pm$ 0.5	36 $\pm$ 0.8	41.3 $\pm$ 1.2	41.5 $\pm$ 0.2
<i>24 h after treatment</i>				
$G_1$	55.5 $\pm$ 0.6	68.6 $\pm$ 0.7 *	56.6 $\pm$ 0.6	65.4 $\pm$ 0.4 *
S	13.8 $\pm$ 0.5	7 $\pm$ 1.2 *	12.9 $\pm$ 0.4	9.7 $\pm$ 0.8 *
$G_2/M$	30.6 $\pm$ 0.1	24 $\pm$ 0.4 *	29.9 $\pm$ 0.2	25.6 $\pm$ 0.1 *



**MELATONIN  
PRETREATMENT  
BEFORE RADIATION  
SENSITIZES BREAST  
CANCER CELLS TO  
THE IONIZING  
EFFECTS OF  
RADIATION**

**p53**



**Decreasing tumoral  
estrogen production:**  
inhibition of aromatase  
and sulfatase activity and  
expression.

**Decreasing cell  
proliferation.**

**Inducing cell cycle  
arrest.**

**Down-regulating RAD-51  
and DNA-PKcs, two proteins  
involved in double-strand  
DNA break repair.**