

LDR and its potential application in clinics

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Gene Burch

LDR effects

Hormesis

Adaptive response

Bystander effects



LDR effects

Hormesis

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ELSEVIER

**EXPERIMENTAL
HEMATOLOGY**

Experimental Hematology 32 (2004) 1088–1096

Low-dose radiation (LDR) induces hematopoietic hormesis: LDR-induced mobilization of hematopoietic progenitor cells into peripheral blood circulation

Wei Li^a, Guanjun Wang^a, Jiuwei Cui^a, Lu Xue^a, and Lu Cai^{b,c}

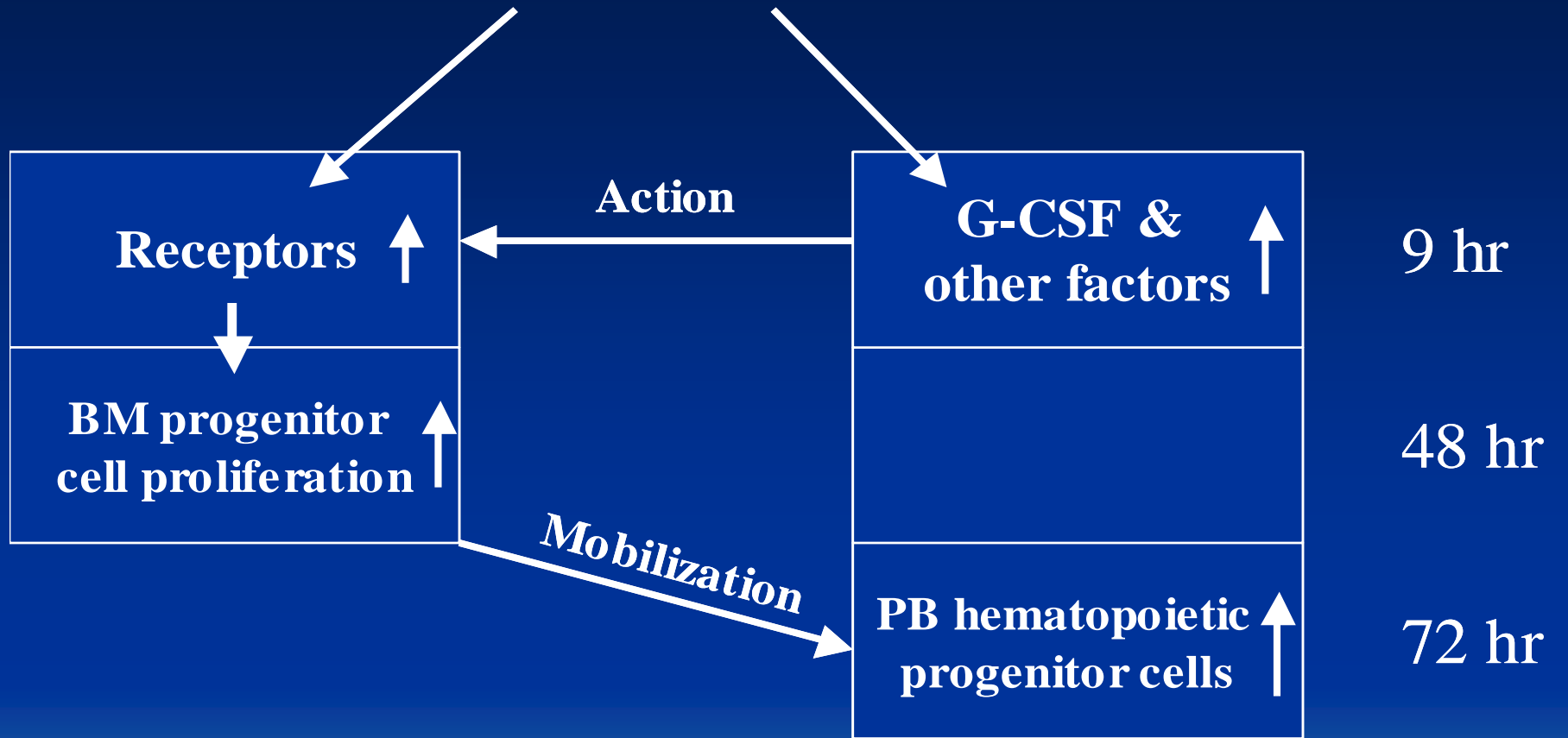
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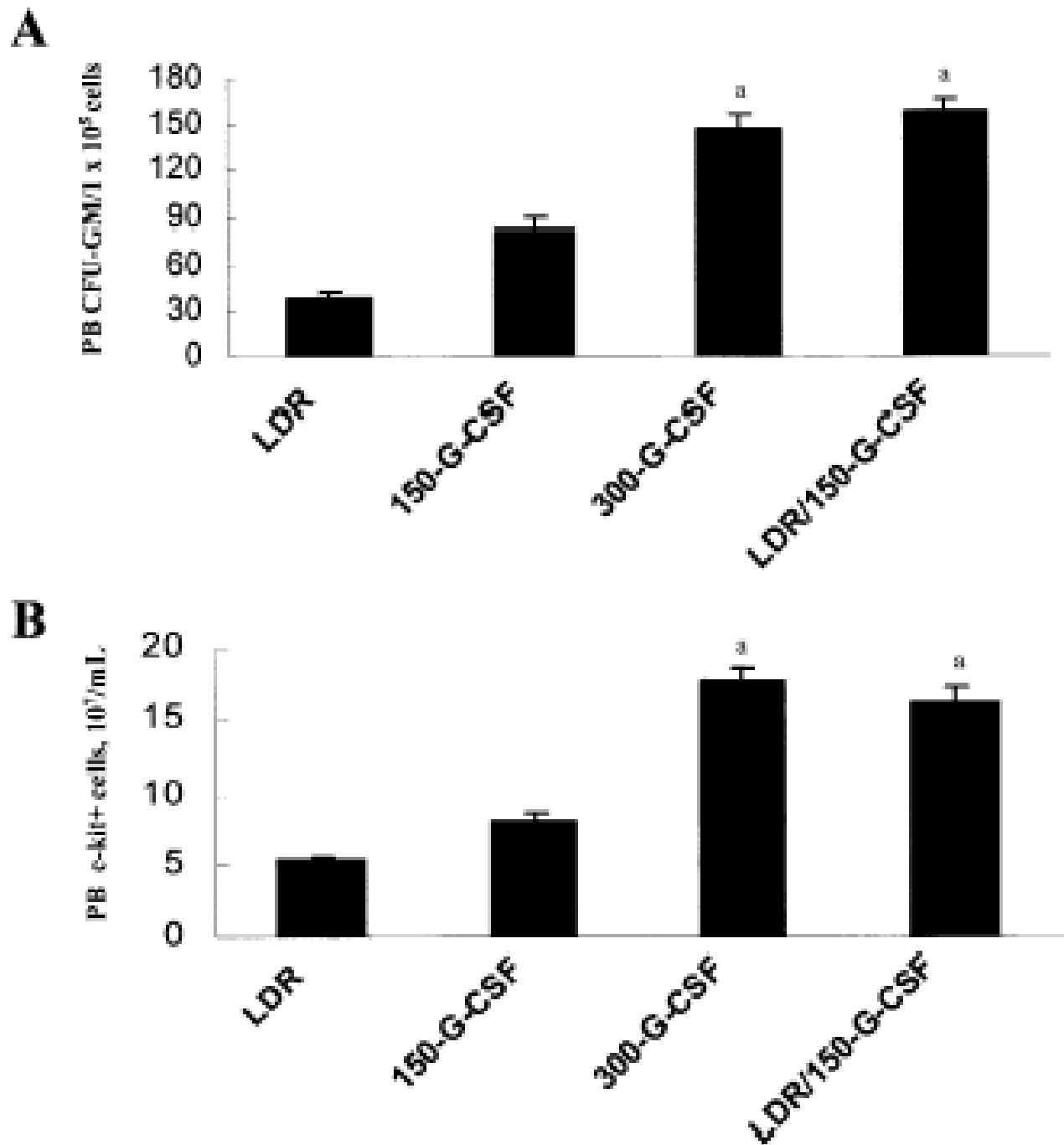
LDR



Bone marrow

Peripheral Blood

Time



- LDR (75 mGy X-rays)/ 12 hr
- G-CSF at 300 $\mu\text{g}/\text{kg}/\text{day}$ every 12 hr for three days
- 150 $\mu\text{g}/\text{kg}/\text{day}$ G-CSF every 12 hours and then LDR once
- CFU-GM formation from peripheral mononuclear cells (A)
- C-kit⁺ numbers in peripheral mononuclear cells (B)

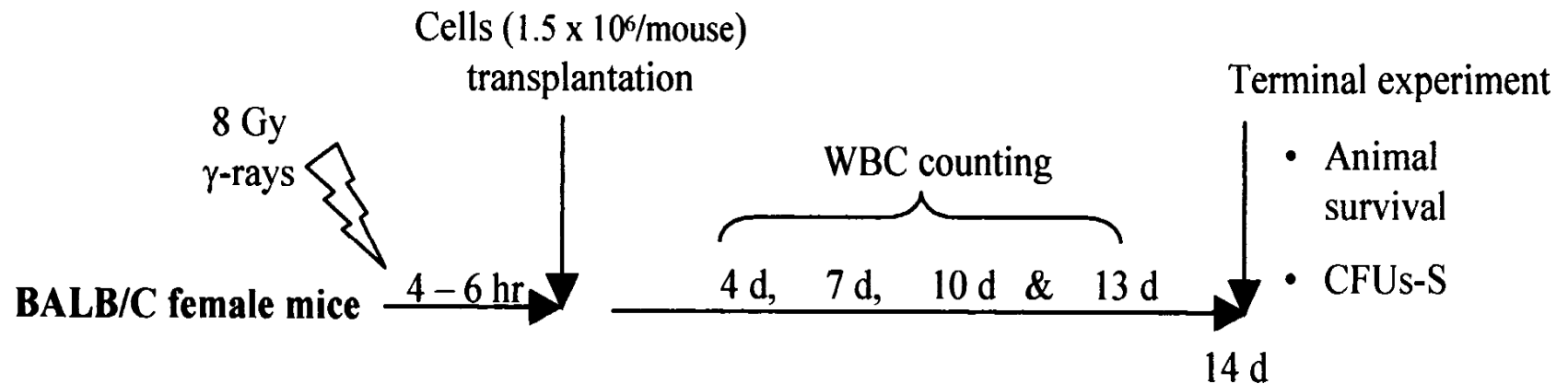
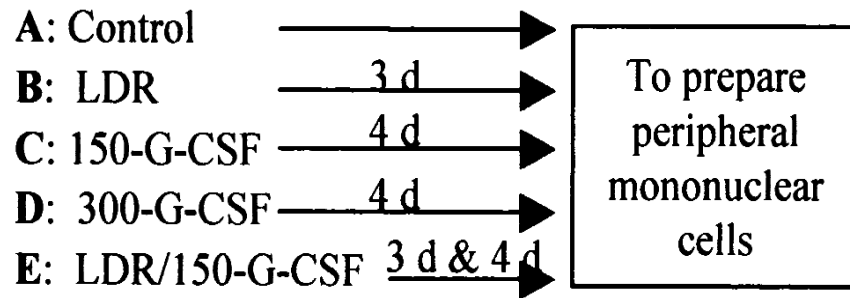
BALB/C male mice

Figure 1. Scheme of experimental procedures for evaluation of LDR-mobilized HPCs. LDR: 75 mGy X-rays; 150-G-CSF or 300-G-CSF: 150 or 300 $\mu\text{g}/\text{kg}/\text{day}$ G-CSF administration; LDR/150-G-CSF: combining treatments of LDR and 150 $\mu\text{g}/\text{kg}/\text{day}$ G-CSF administration. WBC: white blood cells. “3 d” in **B** group indicates that mice were sacrificed 3 days after LDR; “4 d” in **C** and **D** groups indicates that mice were consecutively administrated with G-CSF for 4 days and then sacrificed; “3 d & 4 d” in **E** group indicates that mice were consecutively administrated with G-CSF for 4 days and irradiated with LDR at 3 days prior to the last administration of G-CSF, and sacrificed after the last G-CSF administration. For BALB/C female mice, “4 d, 7 d, 10 d, 13 d, and 14 d” indicate the time (days) after HPC transplantation.

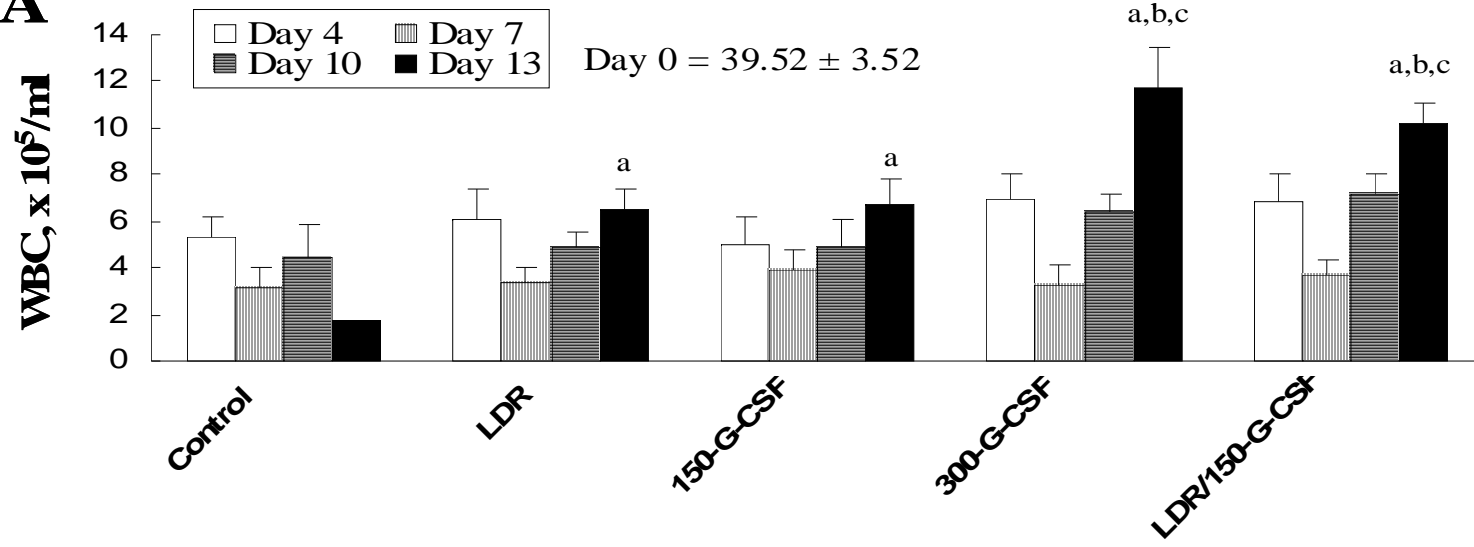
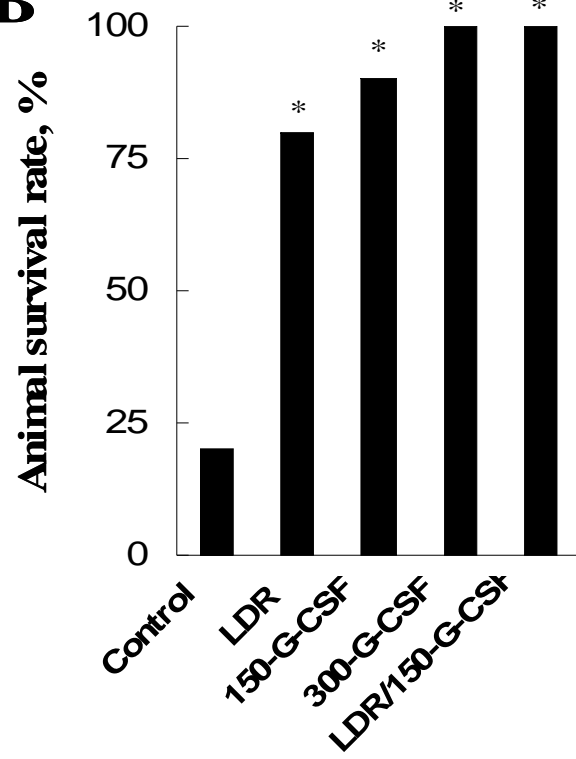
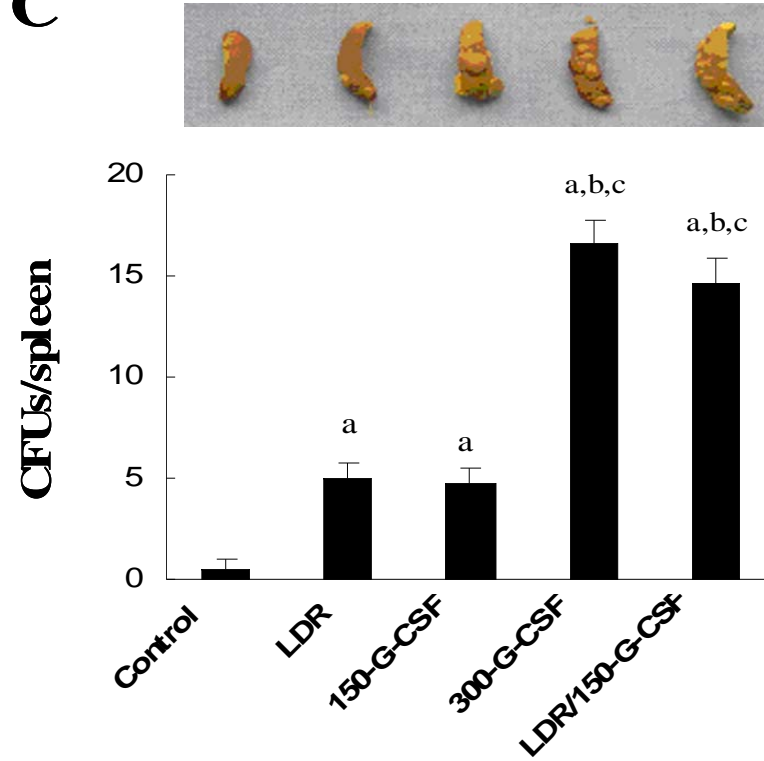
A**B****C**

Table 1 Characteristics of different approaches to mobilize peripheral blood progenitor cells based on reviews (10,21) and the present study.

Approaches	Advantages	Disadvantages
Hematopoietic growth factors such as G-CSF	Efficient	Expensive Bone painful Headache
Chemotherapy such as cyclophosphamide	Efficient	Expensive Toxic *
Combined above two	Efficient	Still expensive Toxic
LDR and G-CSF	Efficient Easier process Inexpensive Co-immunostimulation Multiple factor stimulations Enhancing immune & hematology adaptive response	Unknown yet

* Toxicities are not only the risk of hemorrhagic cystitis, fluid retention, and cardiomyopathy, but also the transient pancytopenia with infection of high dose chemotherapy drug such as cyclophosphamide (21).

Can we directly use LDR into clinics now?

- LDR may not only stimulate normal cell proliferation, but also stimulate the potent tumor cell proliferation or *in situ* tumor cell metastasis.
- LDR may not only enhance normal tissue resistance to subsequent radio- or chemo-therapy-induced side toxicity, but also make tumor cells become radio- or chemo-therapy resistance (drug resistance).



Literature information

Park *et al.* Cell Biol Toxicol. 15 (1999) 111-119.

- A few mouse normal cell lines: lymphocytes (NL), mouse connective tissue cells (L929) and primary mouse keratinocytes (PK)
- A few tumor cell lines: mouse papilloma (line 308) and mouse lymphoma cells (L5178Y-S and EL-4)
- Investigate the difference for LDR-induced adaptive response, as determined by cell survival and apoptosis.
- Adaptive response was induced by pretreatment with 10 mGy X-rays in normal cells such as NL, L929, and PK cell lines, but not in L5178Y-S, EL-4, and line 308 cells for cell survival rate.
- For reduction of apoptosis by pretreatment with LDR was also observed only in normal NL, L929, and PK cells, but not in tumor L5178Y-S, EL-4, and 308 cells.

Our question

Whether or not LDR induces a same hormesis between normal and tumor cells?

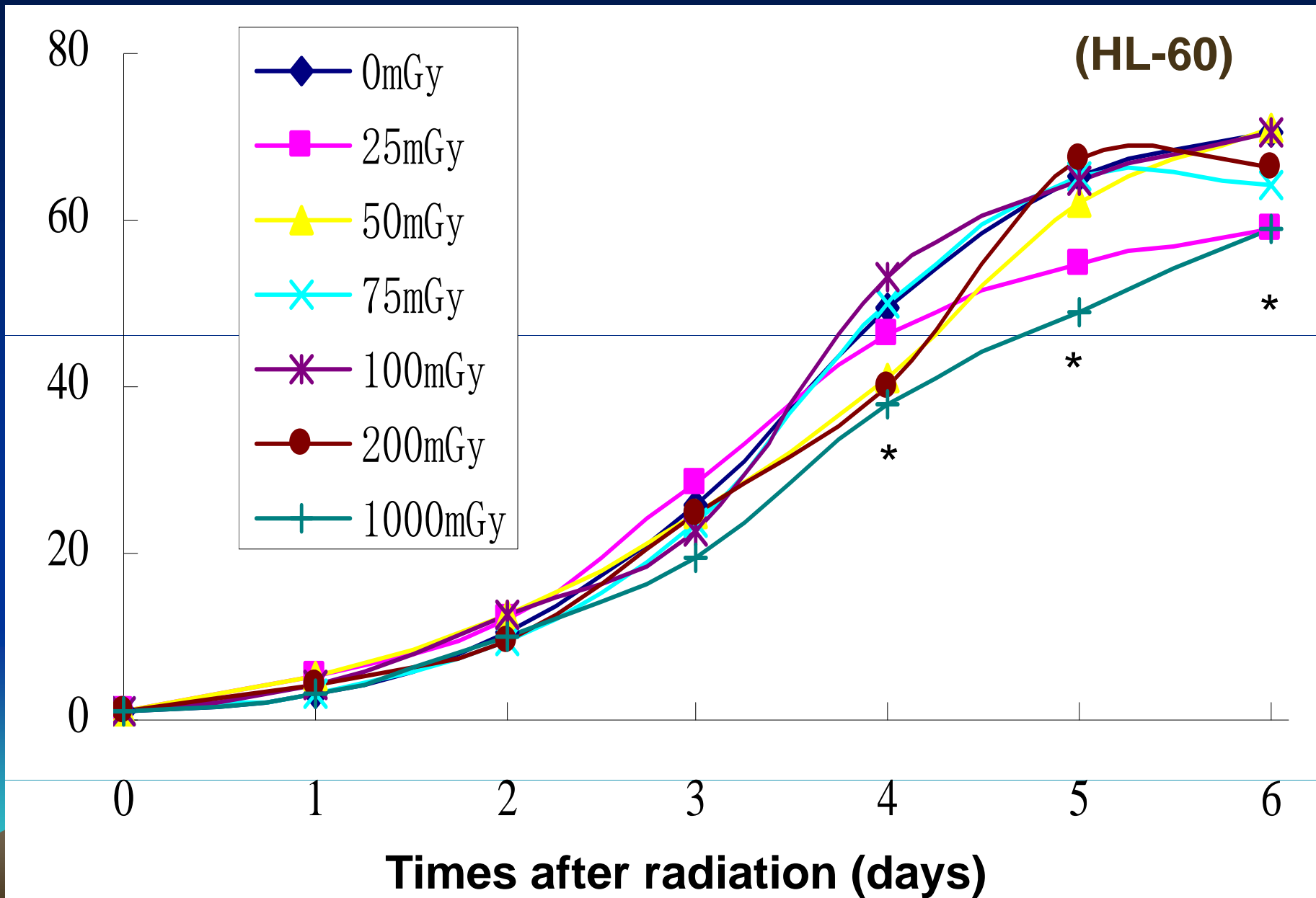
Cell proliferation

- Stimulation
- Adaptive response

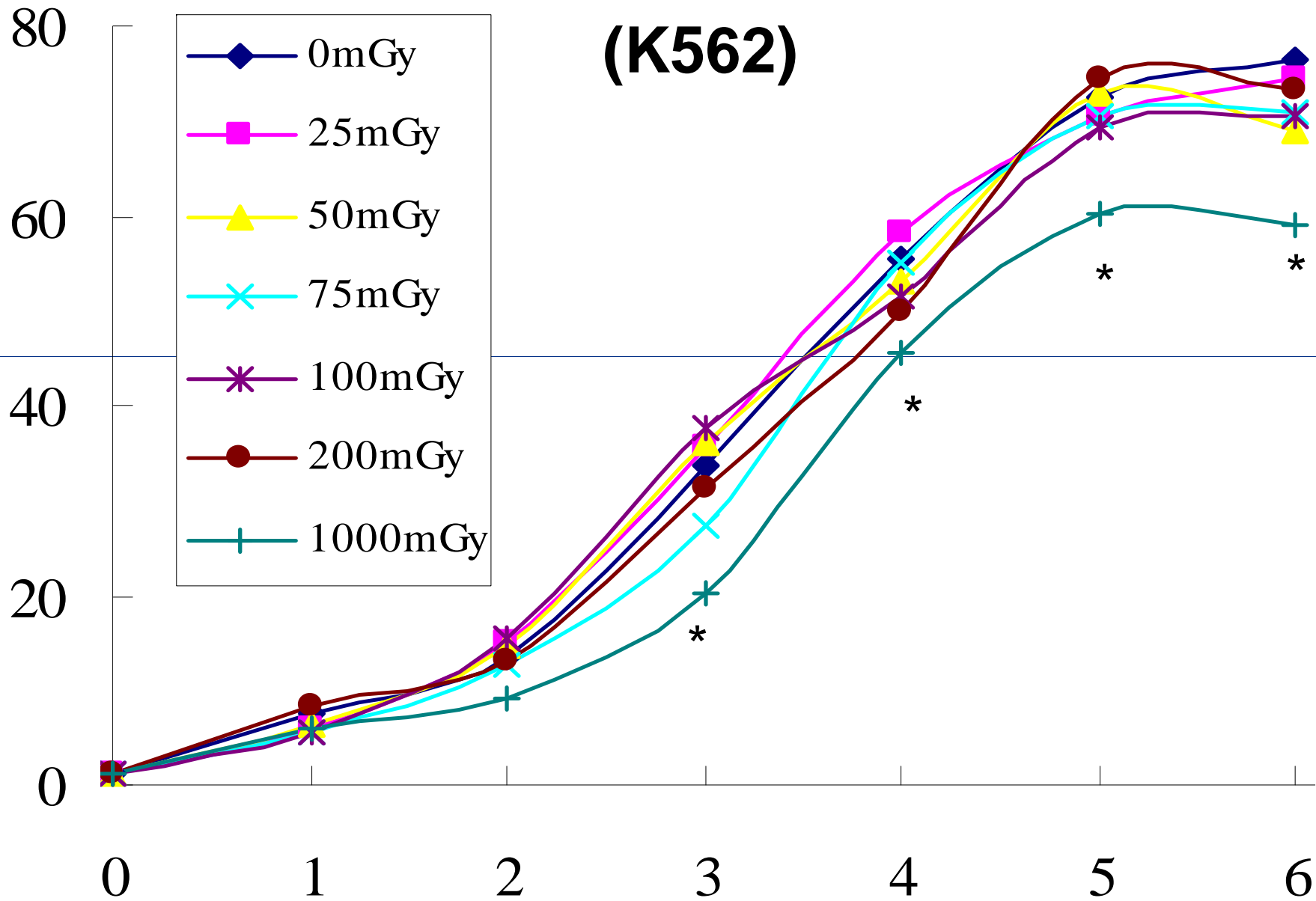


Origin of the human cell lines and their culturing conditions

Cell line	Origin	Medium	FCS
<i>Leukemia cell lines</i>			
K562	Erythroleukemia cell line	IMDM	10%
HL-60	Acute promyelocytic leukemia cell line	IMDM	20%
<i>Solid tumor cell lines</i>			
NCI-H446	Small cell lung carcinoma cell line	RPMI	10%
BEL7402	Hepatocellular carcinoma cell line	RPMI	10%
U251	Glioma cell line	IMDM	10%
IICT-8	Ileocecal cells	RPMI	10%
HeLa	Cervical tumor cell line	RPMI	10%
<i>Normal cell lines</i>			
MRC-5	Lung fibroblast cell line	EMEM	10%
HL7702	Liver cell line	3:1 DMEM:F12	10%
293T	Embryonic kidney cell line	DMEM	10%
6550 HLEPiC	Lens epithelial cell line	DMEM	20%

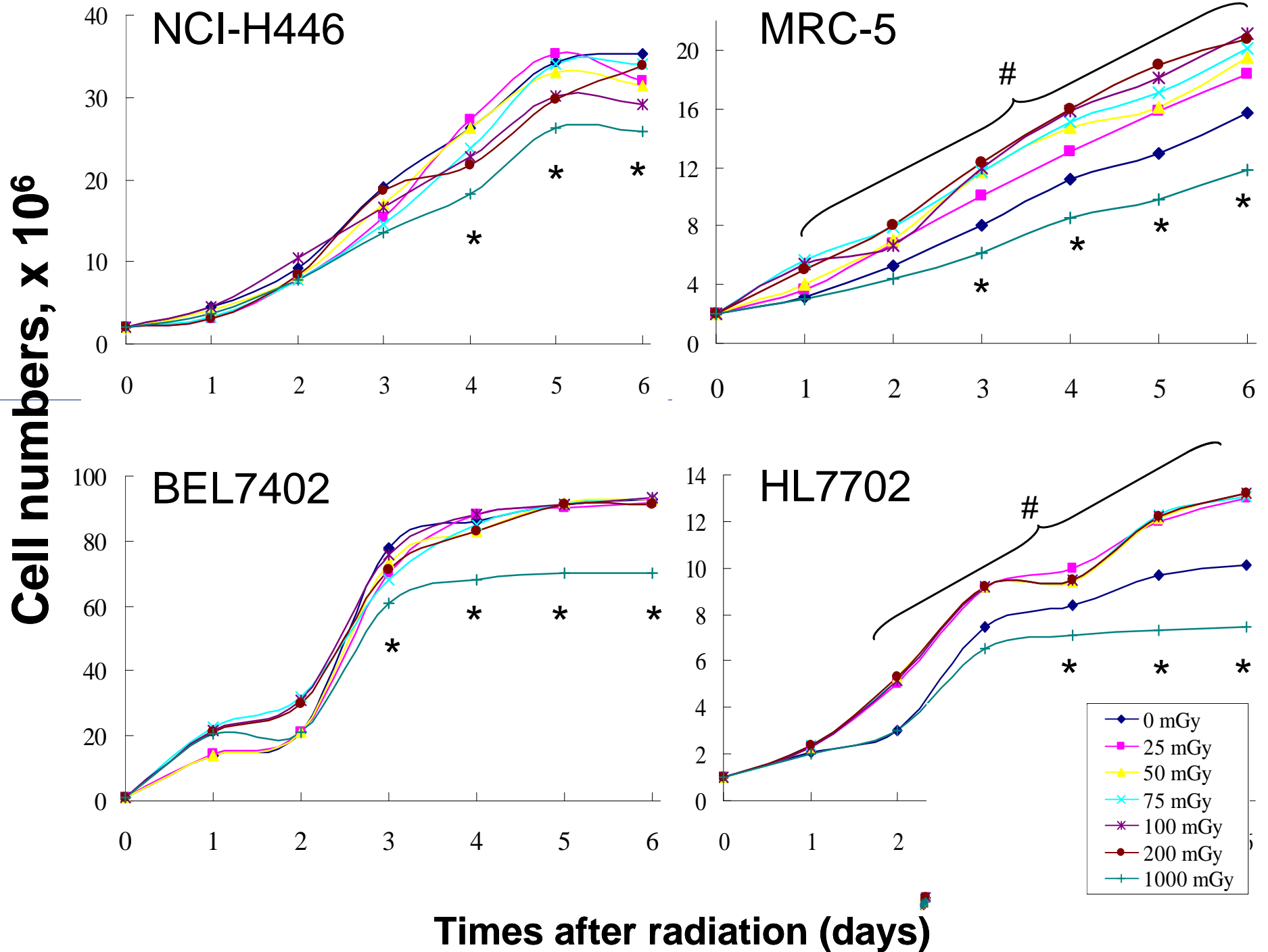


(K562)



Origin of the human cell lines and their culturing conditions

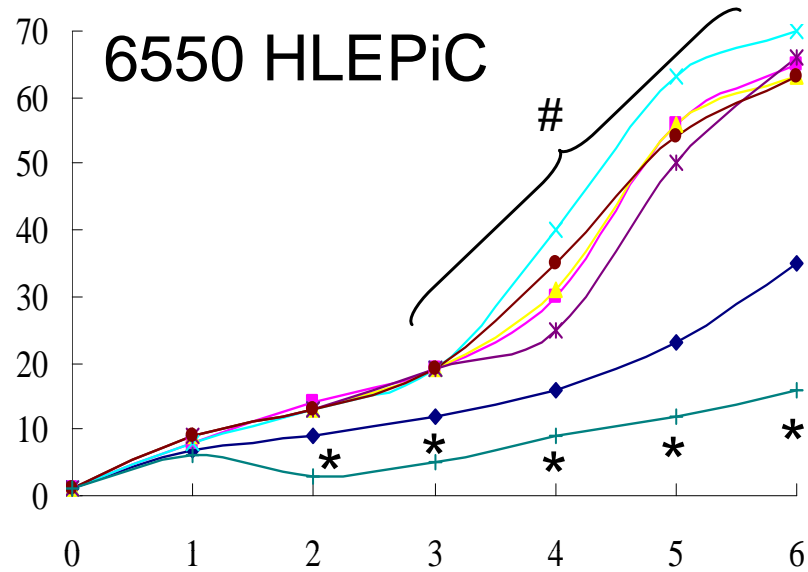
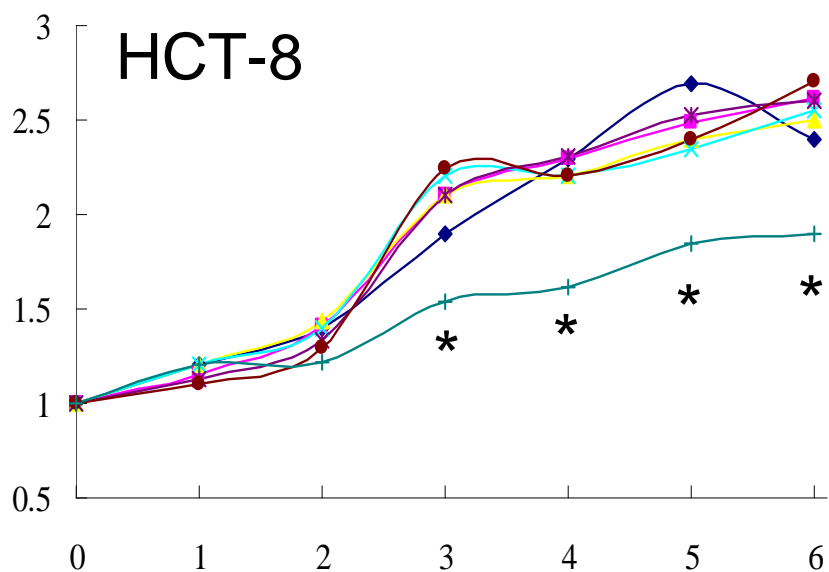
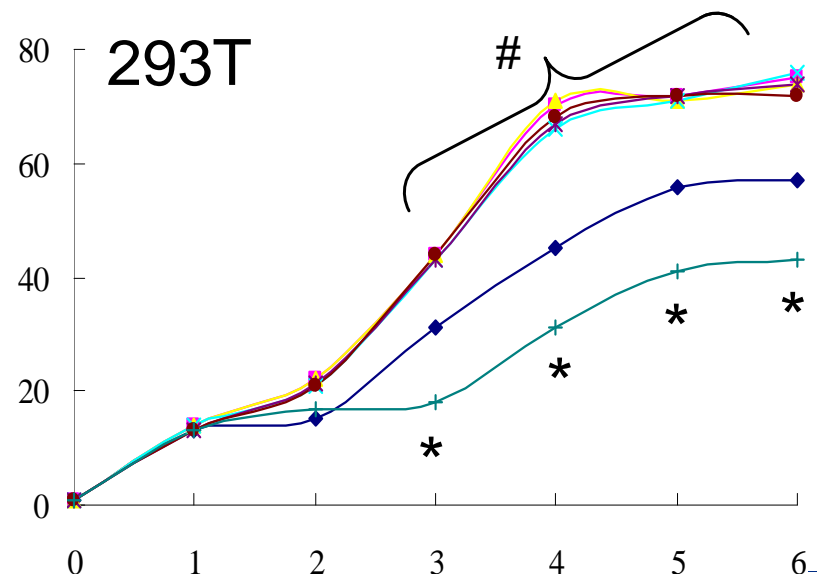
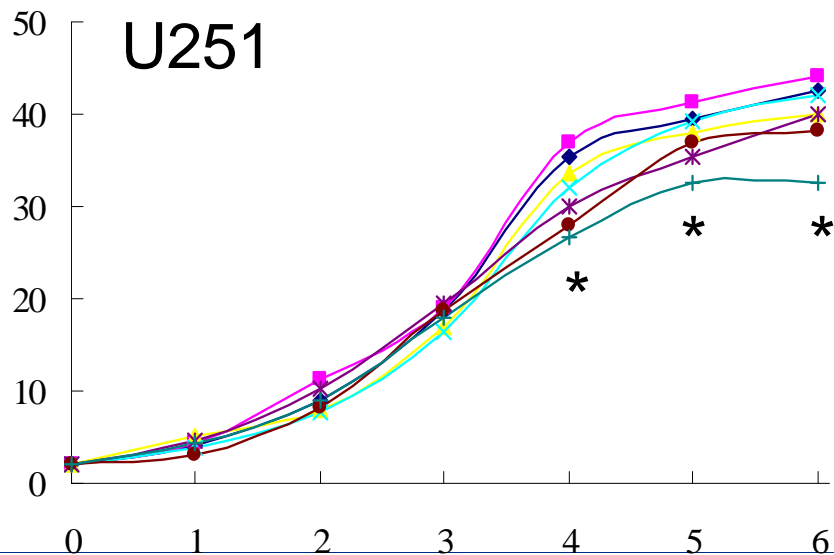
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Cell numbers, x 10⁶



Times after radiation (days)

In vitro studies using several human tumor cells indicate the absence of LDR-induced stimulation of cell proliferation



**In vitro studies using several human
tumor cells indicate the absence of
LDR-induced stimulation of cell
proliferation**

?

In vitro = in vivo

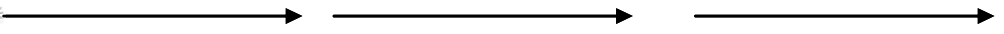


Day 0 5 10 15 20 25 30 35

Sham



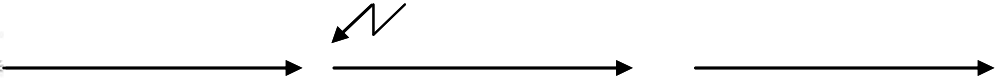
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75 mGy/Post-d15



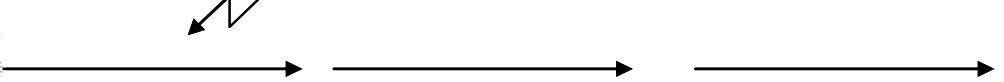
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75 mGy/Post-d10



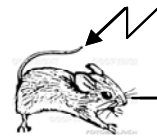
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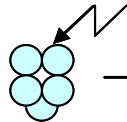
75 mGy/Pre-d0



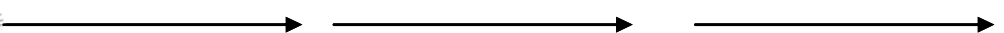
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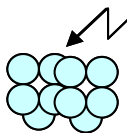
75 mGy/Cells(6,000,000)



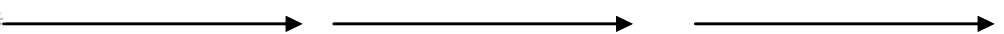
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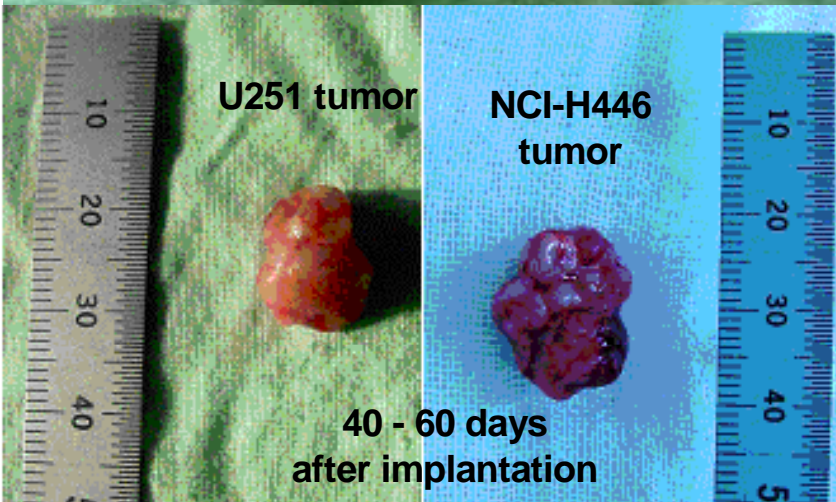
75 mGy/Cells(10,000,000)



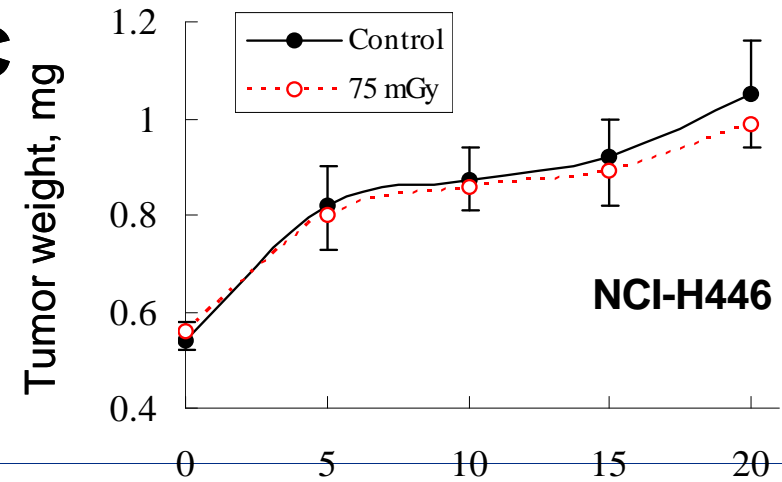
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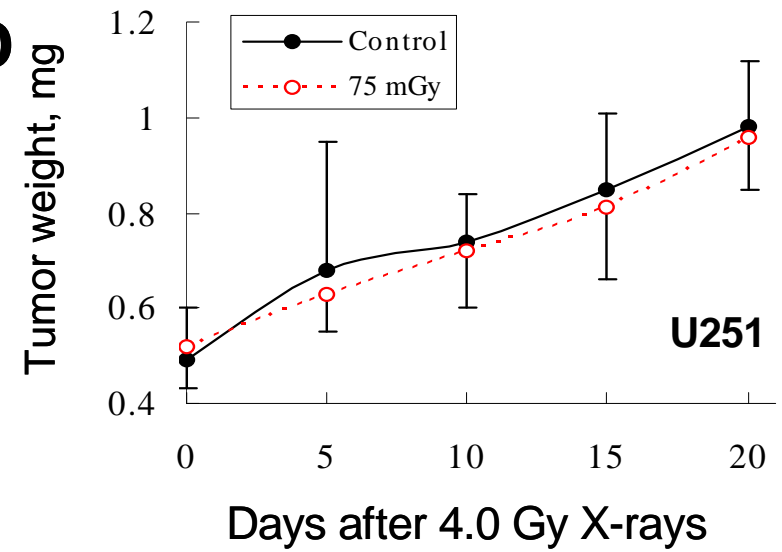
B Tumor-bearing nude mouse model

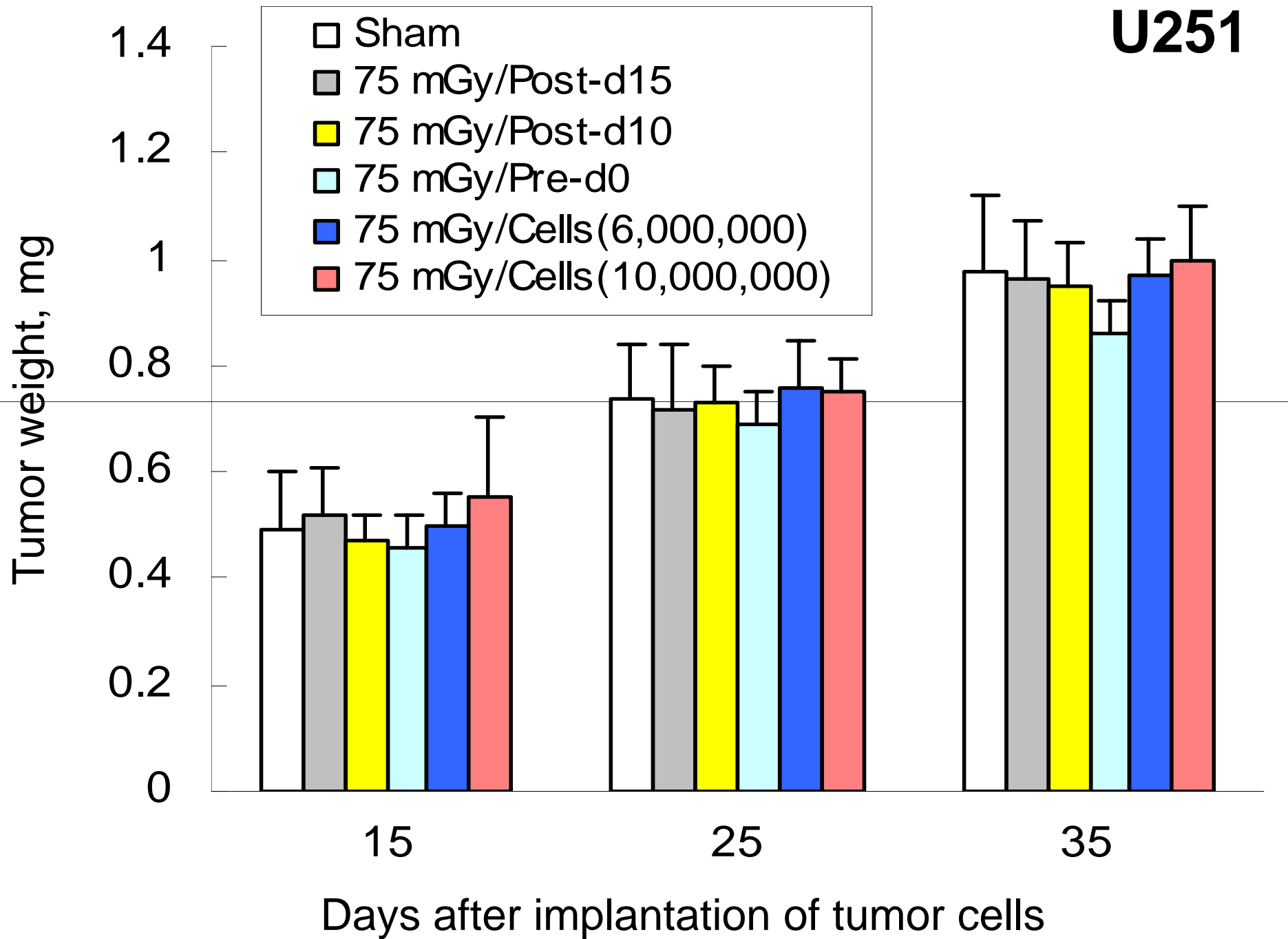


C

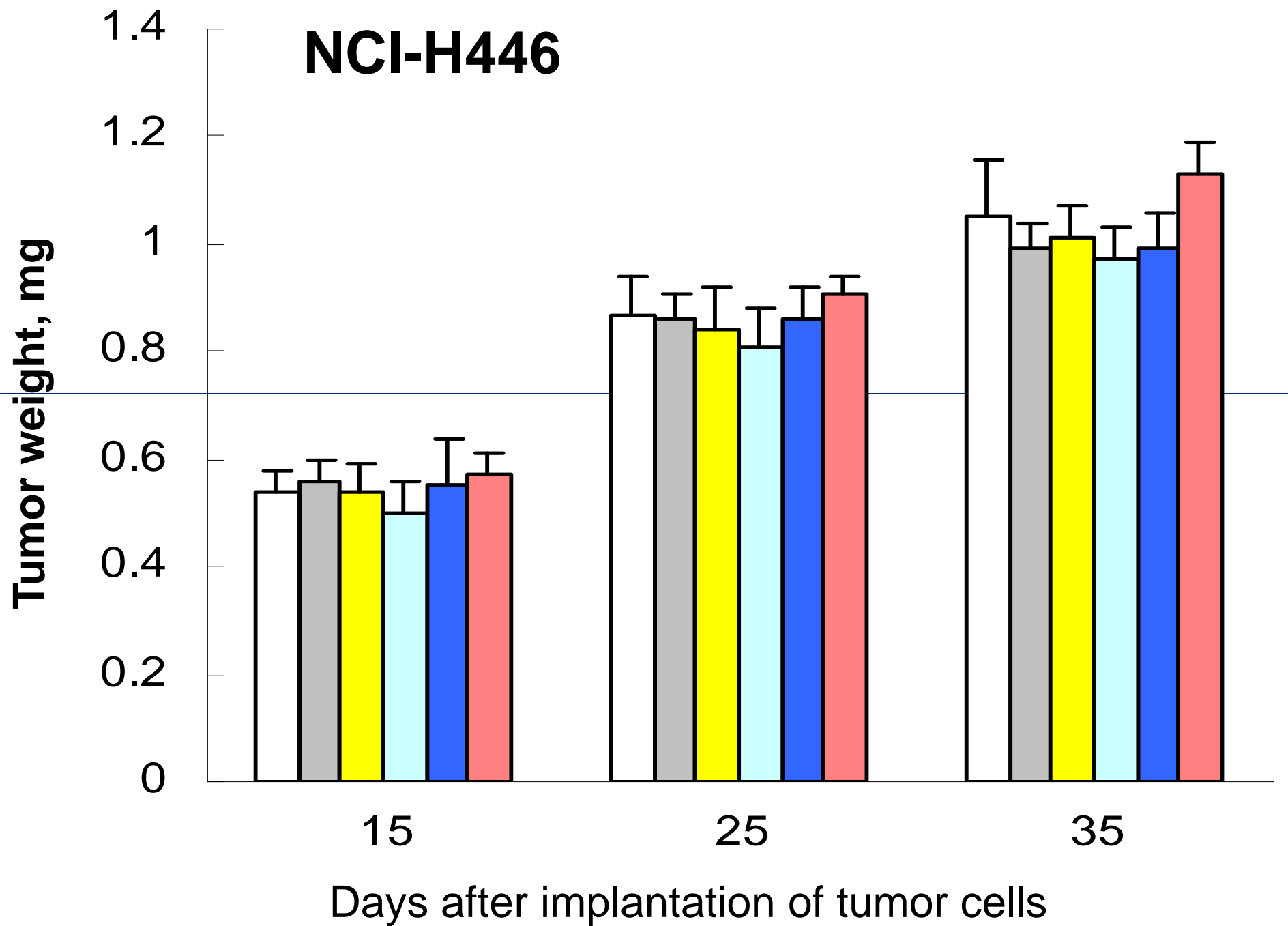


D





NCI-H446



No hormesis in human tumor
cells in response to LDR

In vitro & in vivo



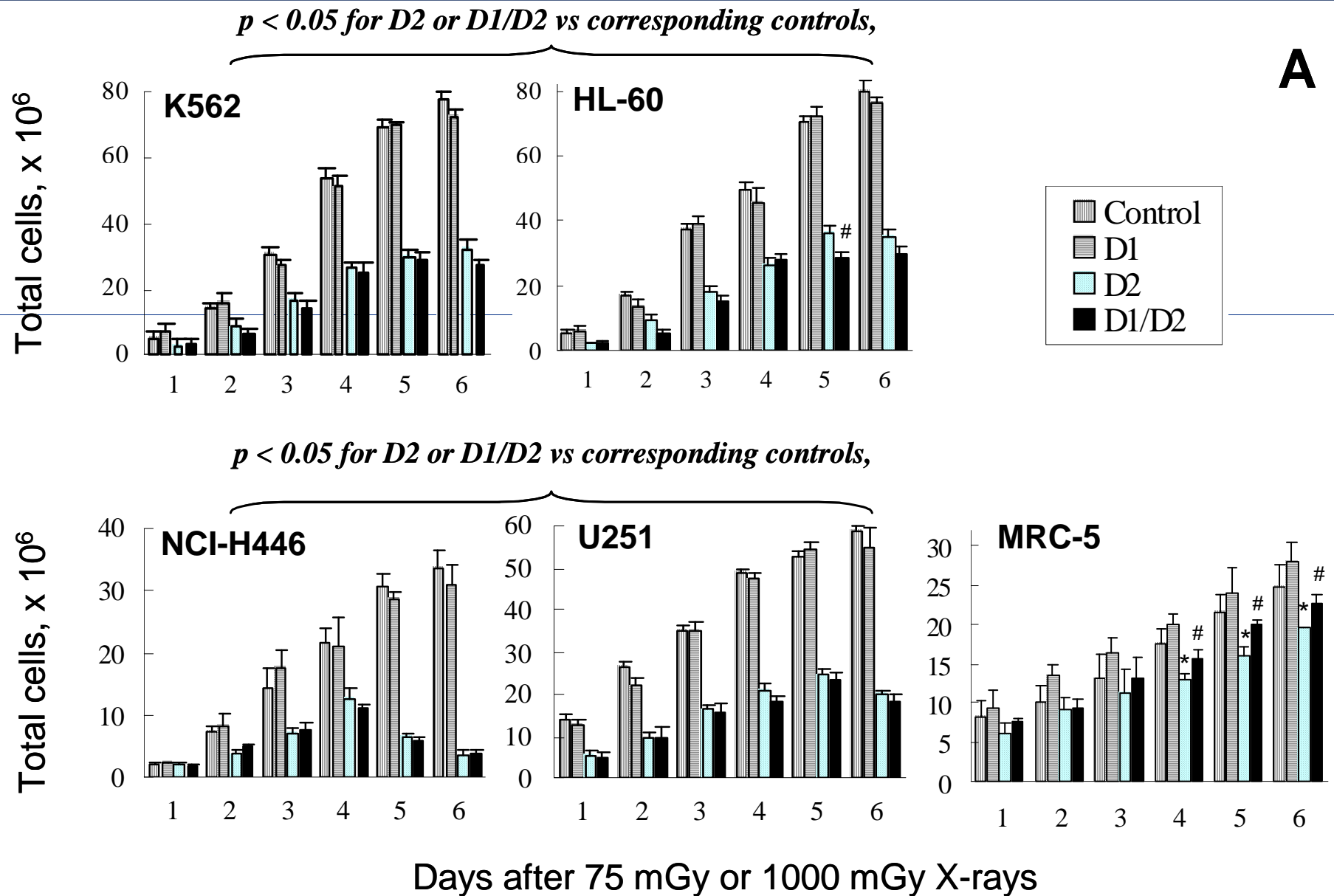
No hormesis in human tumor cells in response to LDR

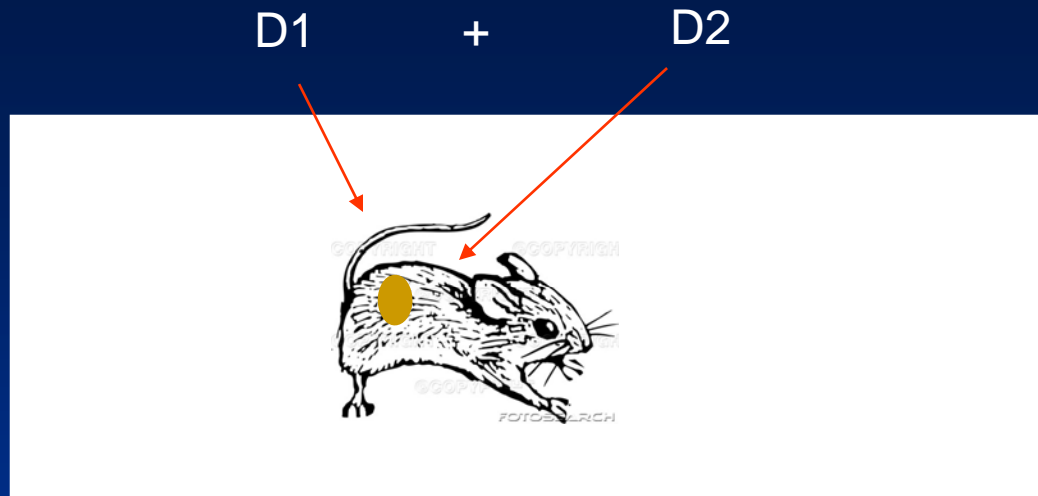
In vitro & in vivo

Whether LDR induces same adaptive response between normal and tumor cells?



75 mGy (D1) - 12 hr - 1 Gy and then count cells at



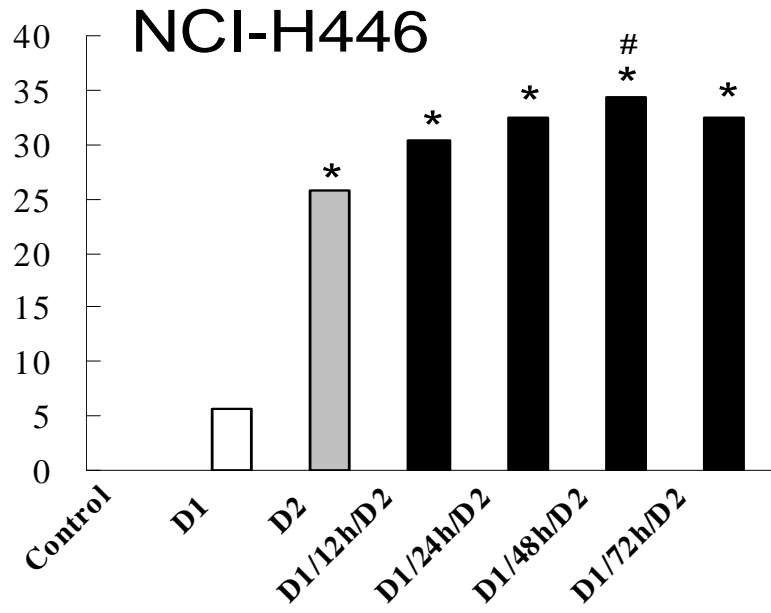


When mice were killed on day 20 after D2 therapy,

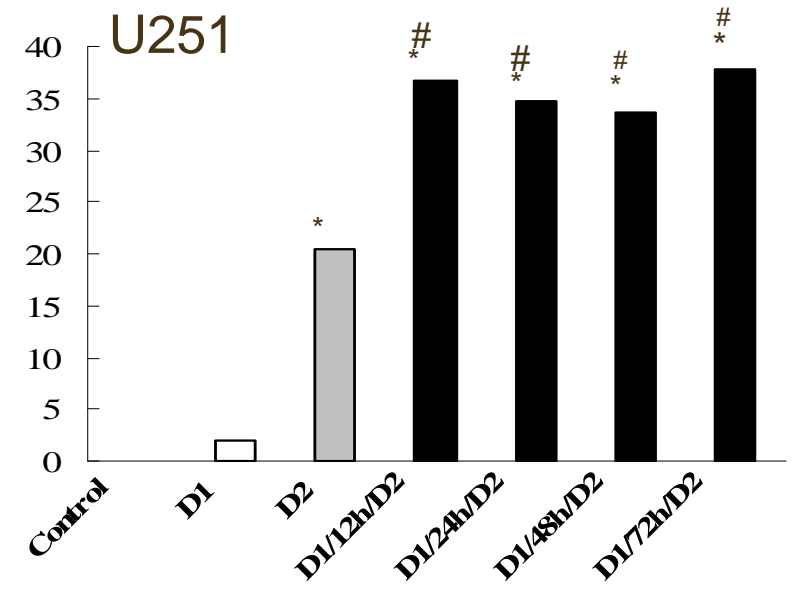
tumor inhibition (%) was calculated:

$$\frac{\text{tumor volumes in sham} - \text{tumor volume in irradiated groups}}{\text{tumor volume in sham.}} \times 100$$

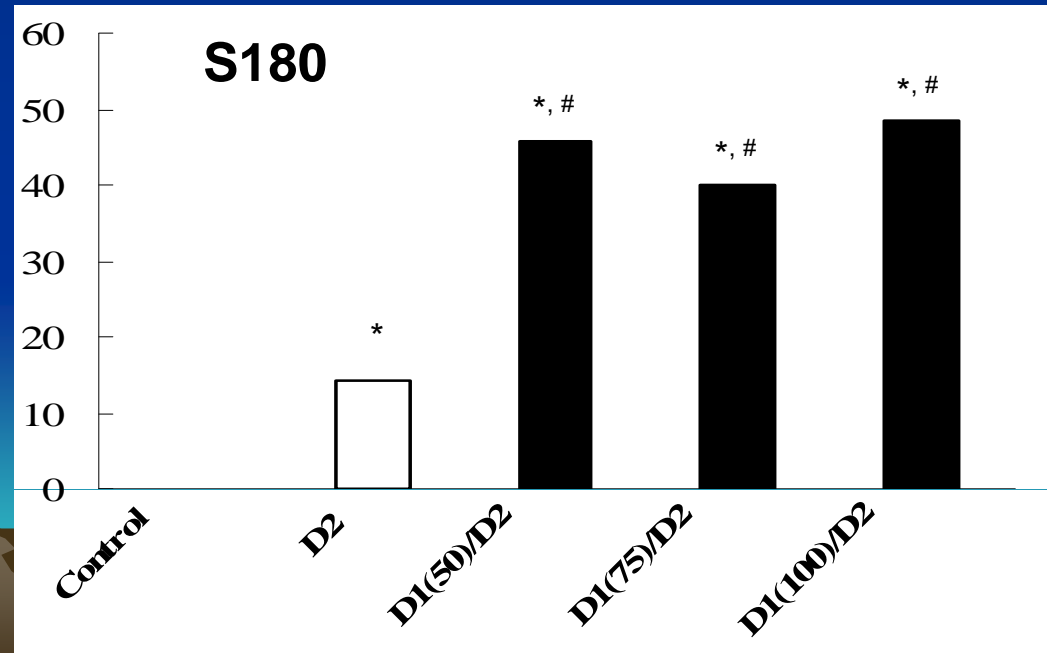
Tumor inhibition, %



Tumor inhibition, %



Tumor inhibition, %



Nude mice

C57BL/6J



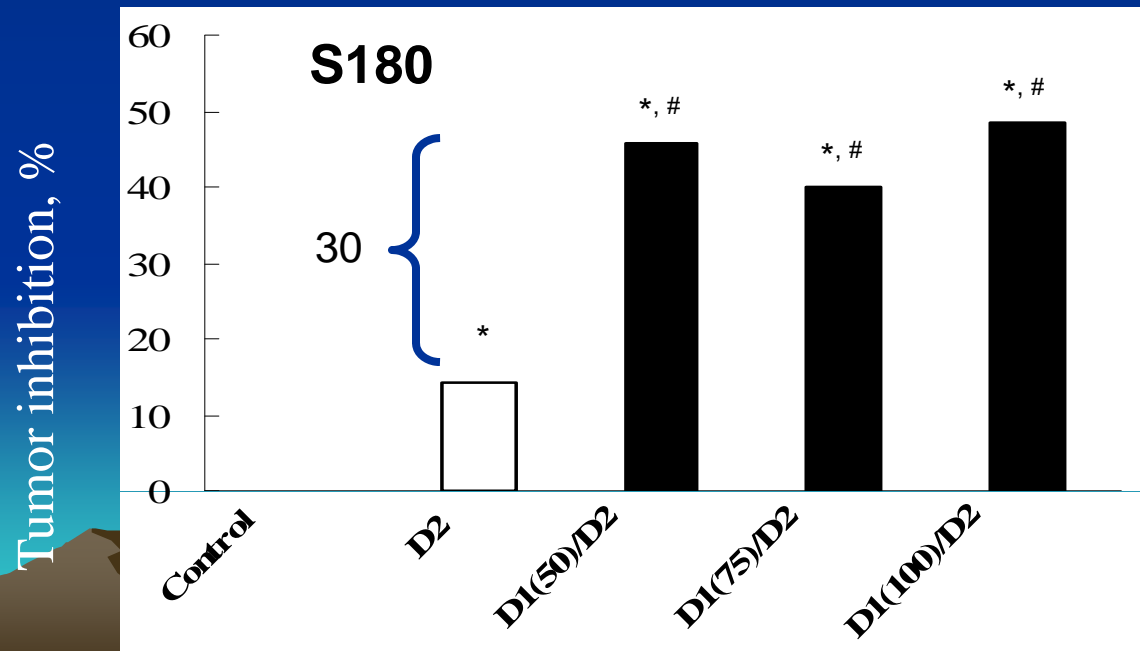
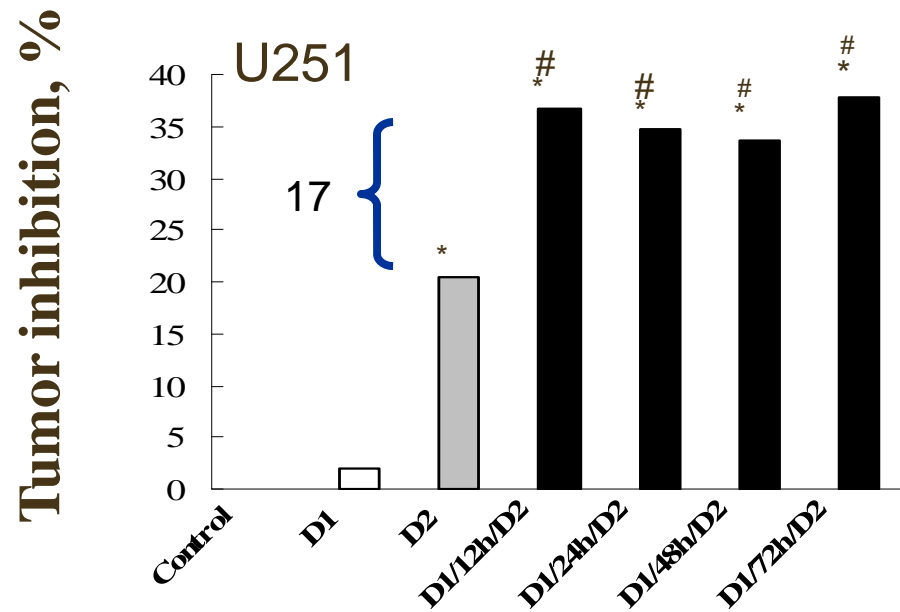
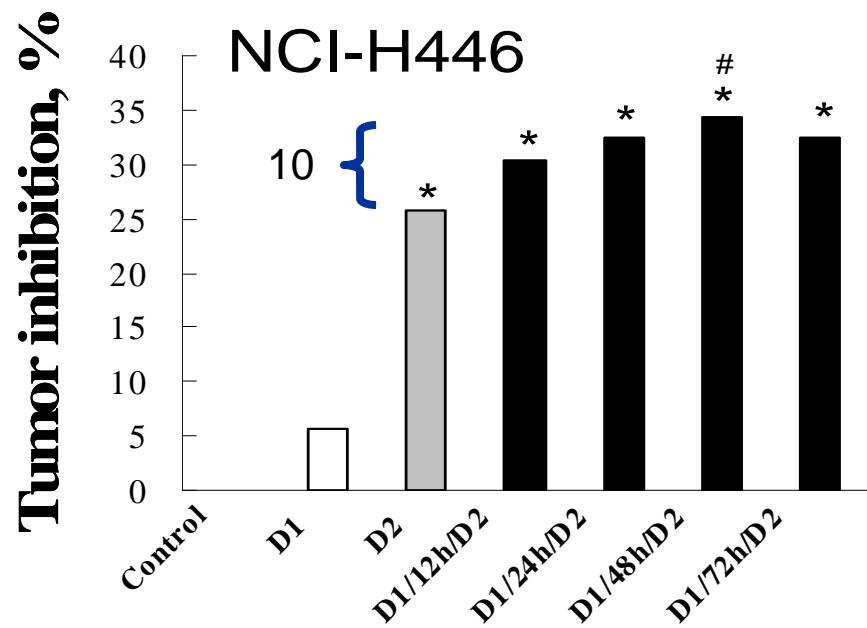
A few in vivo studies

- Cheda A et al. Single low doses of X rays inhibit the development of experimental tumor metastases and trigger the activities of NK cells in mice.
Radiat Res. 161 (2004) 335-340.
- Anderson RE et al. Radiation-induced augmentation of the response of A/J mice to Sal tumor cells.
Am J Pathol. 108 (1982) 24-37.
- Hashimoto S. et al. The suppression of metastases and the change in host immune response after low-dose total-body irradiation in tumor-bearing rats.
Radiat Res. 151 (1999) 717-724.
- Kojima S. et al. Elevation of glutathione induced by low-dose gamma rays and its involvement in increased natural killer activity.
Radiat Res. 157 (2002) 275-280.

- Whole-body LDR, given to tumor bearing mice before implantation of tumor cells, suppressed tumor growth rate (Cheda et al. 2004; Anderson et al. 1982).
- Exposure of tumor-bearing mice to four doses of 250 mGy X-rays at day 0, 7, 14 and 21 after implantation of tumor cells significantly suppressed tumor cell growth (Hashimoto et al., 1999).
- A whole-body irradiation with 200 mGy, given at day 14 day after the implantation of allogenic hepatoma cells (KDH-8), significantly suppressed the incidence of lung and lymph node metastases (Kojima et al., 2002).

These results suggest the absence and even suppression of the tumor cell proliferation potency.





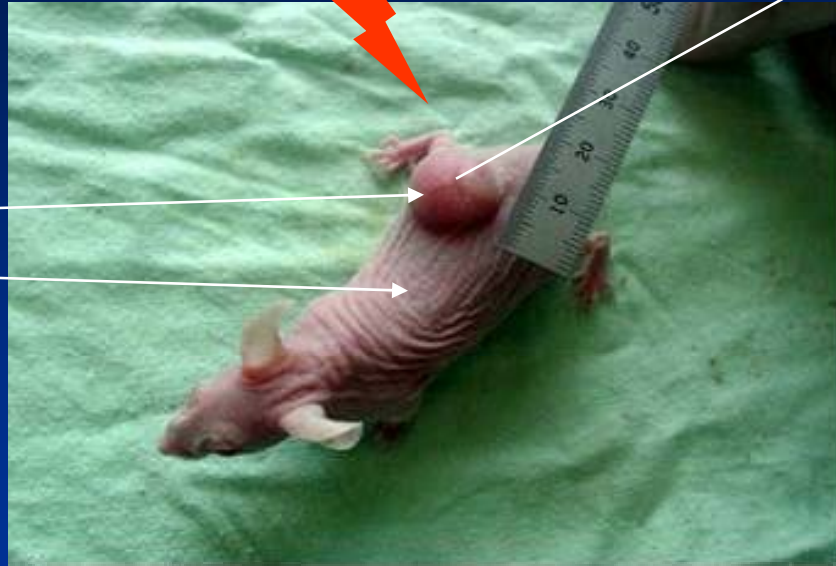
↑
Nude mice

← C57BL/6J

HDR or
chemotherapy

- do not affect tumor sensitivity to radiotherapy or chemotherapy

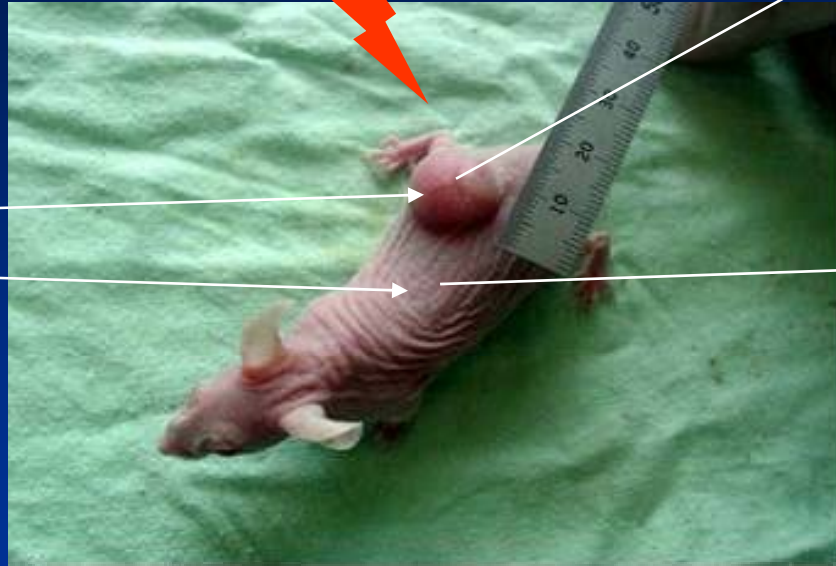
LDR



HDR or
chemotherapy



LDR

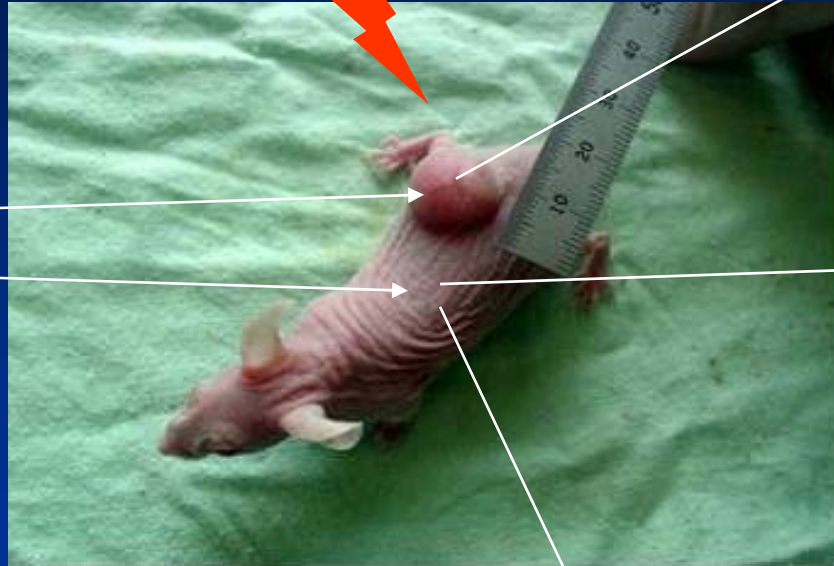


- do not affect tumor sensitivity to radiotherapy or chemotherapy
- LDR-stimulated immuno-function to eliminate the residual cells after radio- or chemo-killed or after surgery

HDR or
chemotherapy



LDR



- do not affect tumor sensitivity to radiotherapy or chemotherapy
- LDR-stimulated immuno-function to eliminate the residual cells after radio- or chemo-killed or after surgery

AR:

- protect the normal tissue from radiotherapy or chemotherapy
- Increase therapeutic dose

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Thank you!

