Acquisition of Radiation Resistant Ability in Non-Irradiated Cells by Secreted Factors from Low Dose Irradiated Cells

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First Observation of Radiation Induced Bystander Effect (RIBE)

[CANCER RESEARCH 52, 6394–6396, November 15, 1992]

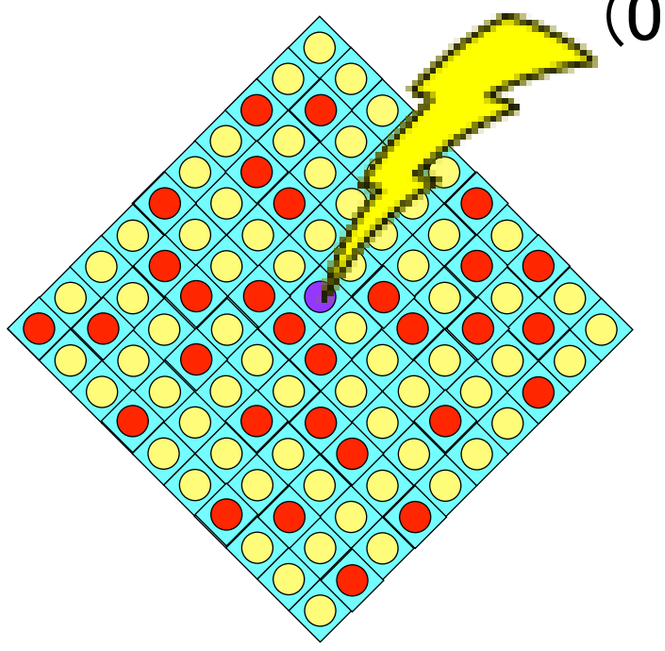
Advances in Brief

Induction of Sister Chromatid Exchanges by Extremely Low Doses of α-Particles[1]

Hatsumi Nagasawa and John B. Little[2]

Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115

α-particle (Pu238)
(0.31 mGy)



: Normal CHO Cells

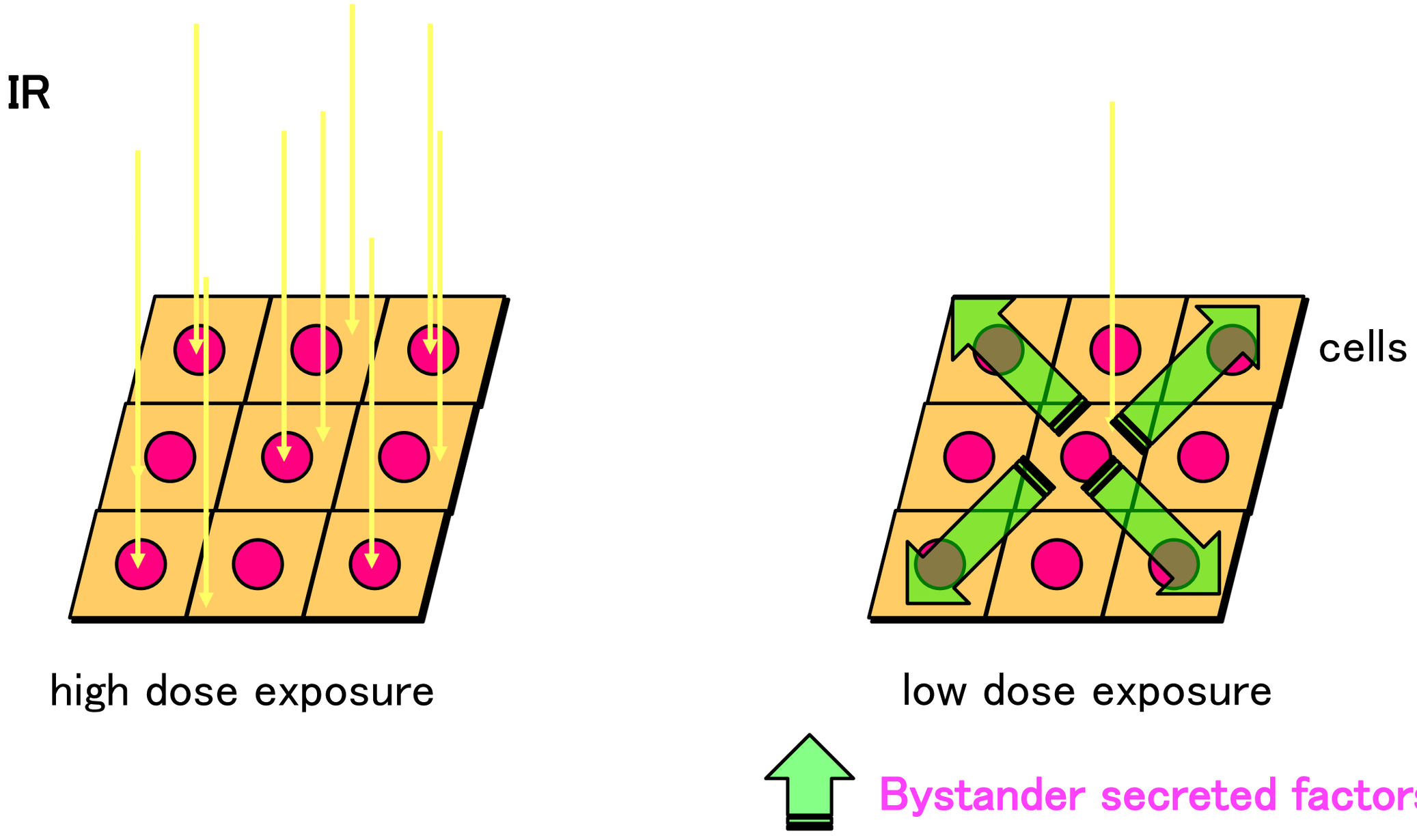
: Irradiated Cells

: SCE Cells

- Less than 1% of cell nuclei were actually traversed by an α-particle.
- 30% of the cells showed an increased frequency of SCE (sister chromatid exchanges)

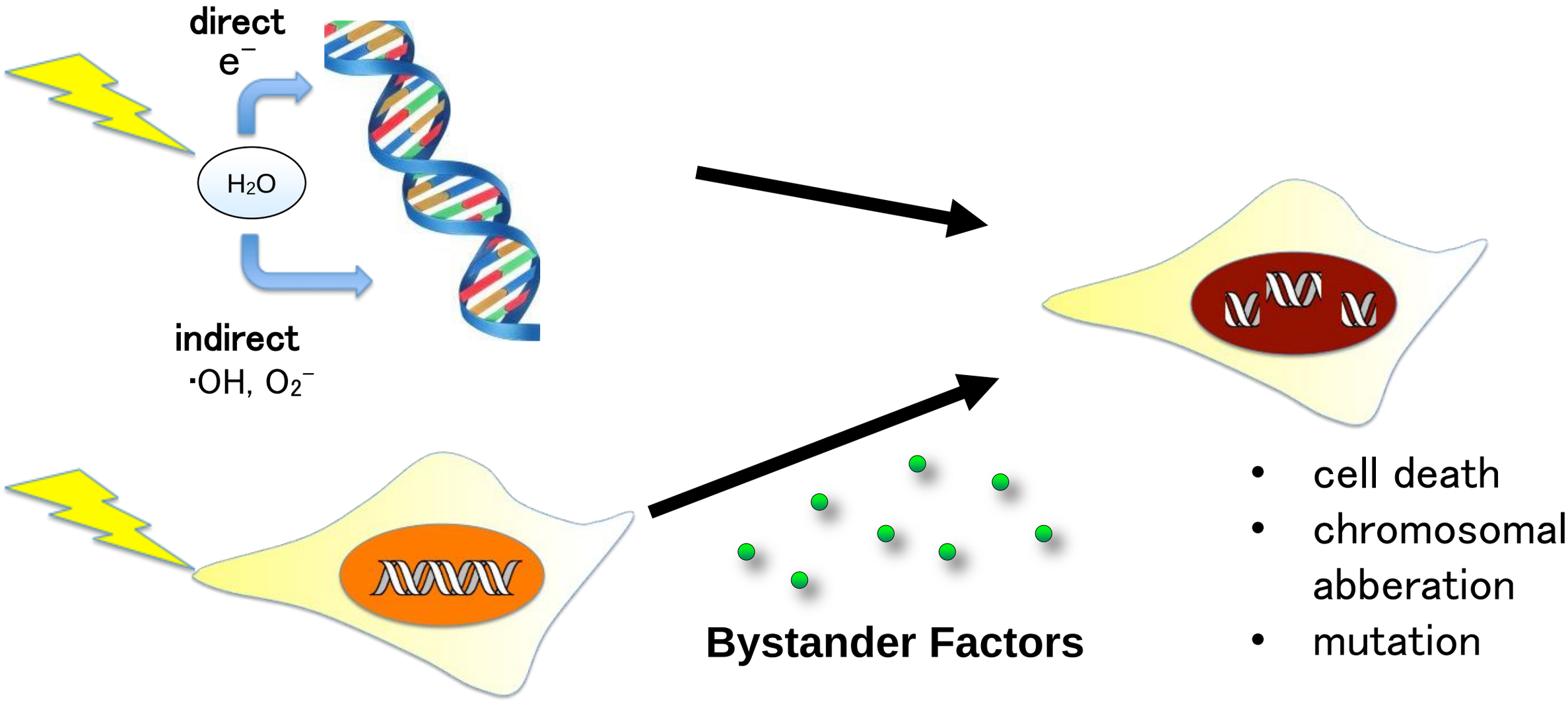
SCEs were induced in non-irradiated cells.

High and low dose exposure to the cells



- Bystander effects is important especially in low dose irradiated cell systems.

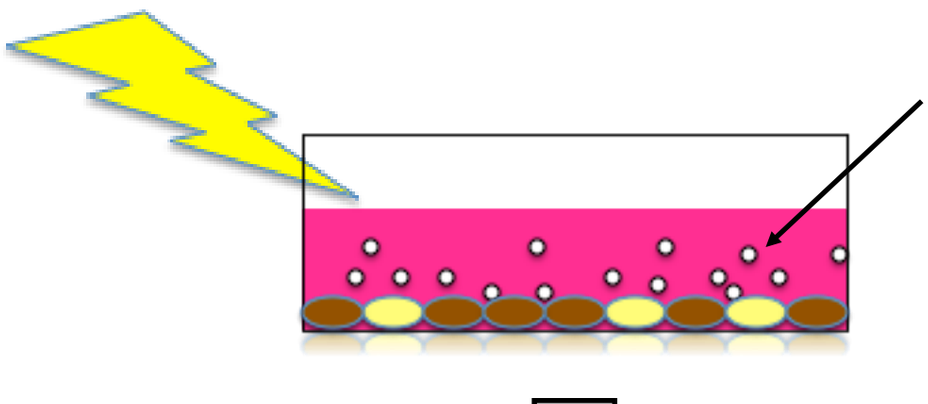
Mixed Radiation Effects



Endpoints are induced not only by direct and indirect effects but also by radiation induced bystander effect.

Bystander Effect Extracted by Medium Transfer (MT) Method

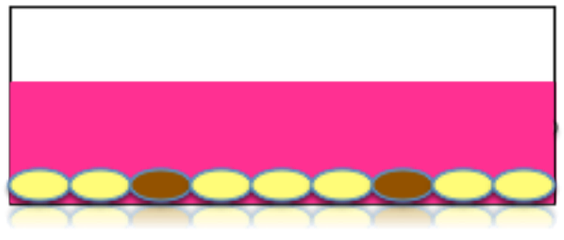
Donor irradiated cells



Soluble Bystander Factors

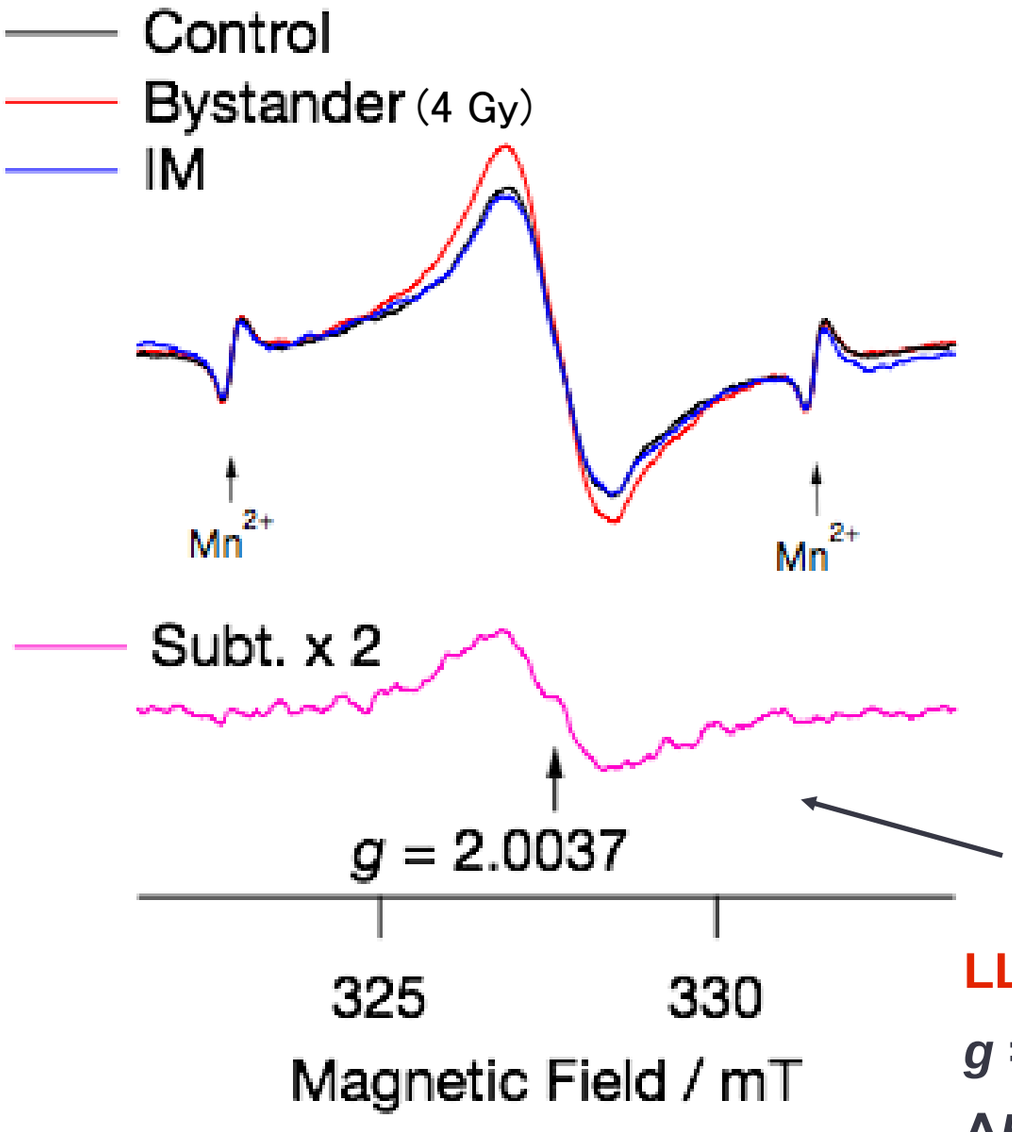
Medium Transfer (MT)

Recipient non-irradiated cells



Endpoints are induced not by radiation but by secreted soluble bystander factors.

ESR spectra of recipient cells
(Chinese Hamster Ovary (CHO) cells)



- **Long-lived Radicals (LLRs)** are induced by the medium transfer (MT) in the recipient cells.

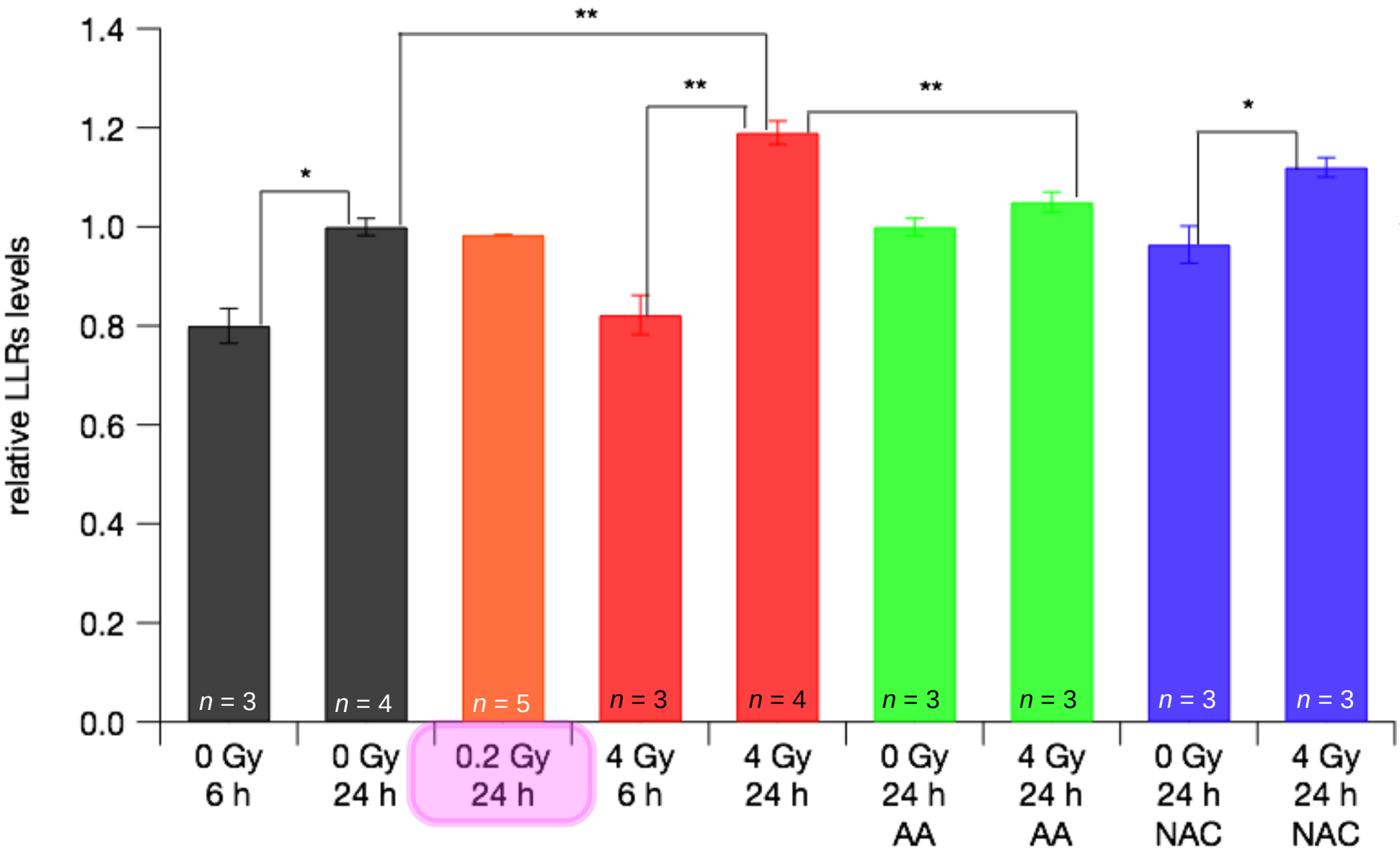
- Good indicator of oxidation degree in cells

LLRs

g = 2.0037

ΔH_{msl} = 1.8 mT

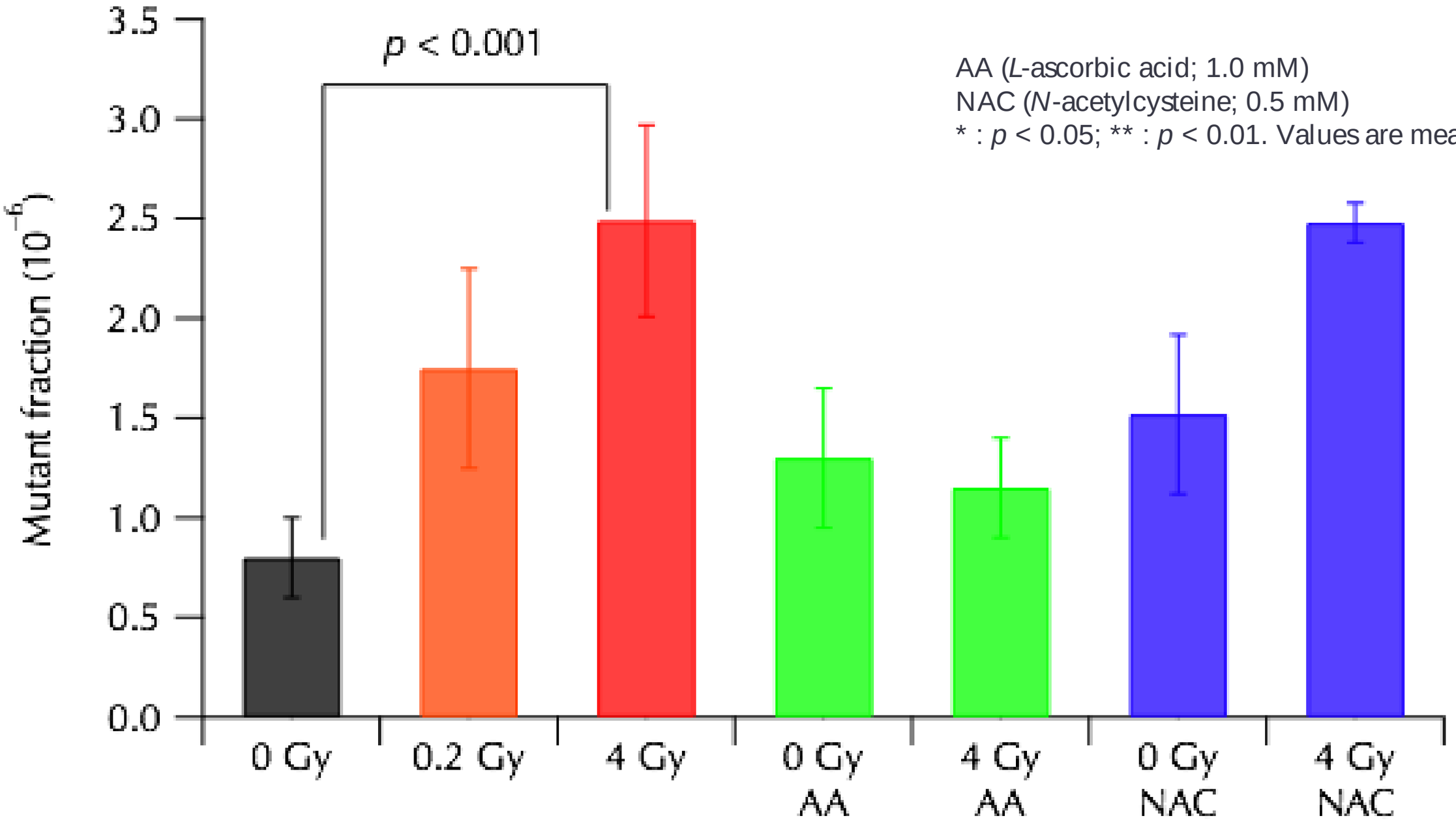
Levels of LLRs in recipient CHO cells by MT. Effect of AA and NAC for the levels.



AA (*L*-ascorbic acid; 1.0 mM)
NAC (*N*-acetylcysteine; 0.5 mM)
* : $p < 0.05$; ** : $p < 0.01$. Values are mean ±S.D.

- Levels of LLRs are increased by MT.
- AA reduced the levels, but NAC did not.

Mutation frequency of recipient CHO cells by MT



AA (*L*-ascorbic acid; 1.0 mM)
NAC (*N*-acetylcysteine; 0.5 mM)
* : $p < 0.05$; ** : $p < 0.01$. Values are mean ±S.D.

- Mutation fraction was increased by MT.
- AA reduced the fraction, but NAC did not.

Free Radical Res. **47** 474–9 (2013)

Purpose of this study

Cell types dependent RIBE

methods of evaluation

Donor cells

Stem cells

cancer cells

Medium Transfer

Recipient cells

normal cells

cancer cells

Bystander Factors

- NO, Cytokines,
- **Exosomes**
  - **vesicles smaller than 150 nm in diameter and are enriched in endosome-derived components.**
  - **distribution of exosomes**

Long-lived radicals (LLRs) produced in recipient cells

LLR levels relate to

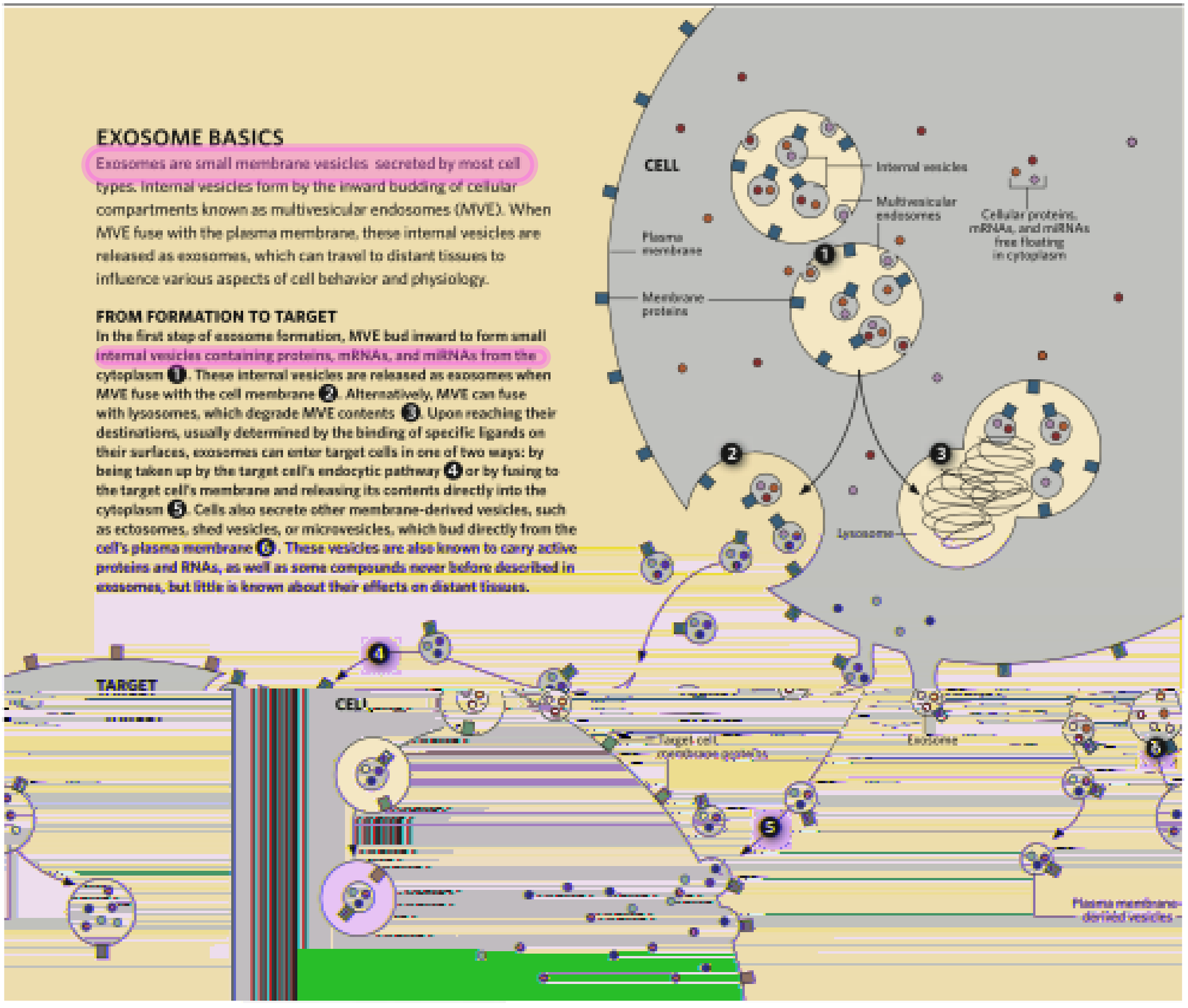
- **oxidation degree in cells**
- **mutation induction**

J. Kumagai and G. Kashino, *Radiat. Chem.*, **89** 2010, pp. 20-24

Exosomes: secreted vesicles and intercellular communications

Clotilde Théry

F1000 Biology Reports 2011, 3:15 (doi:10.3410/B3-15)



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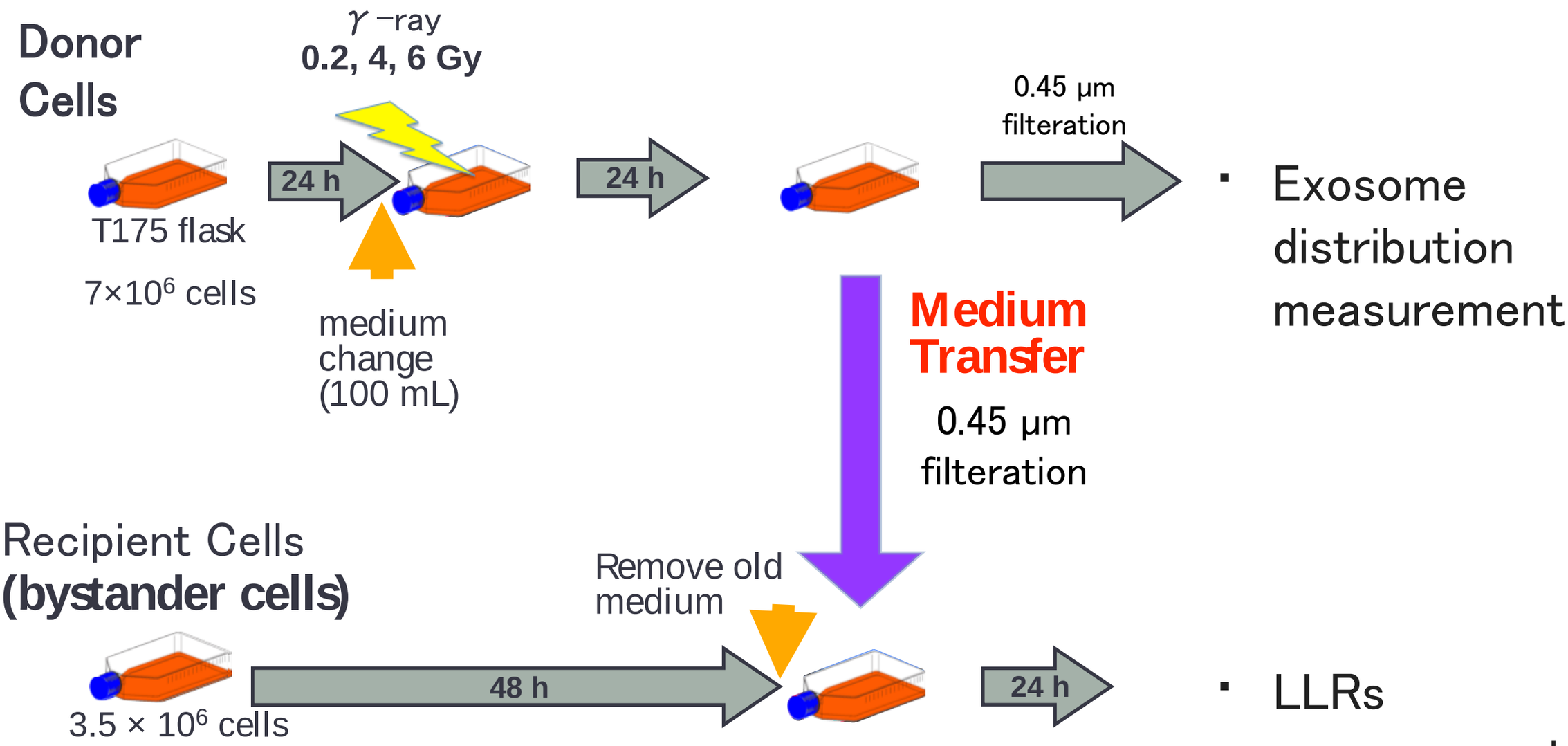
Cell types and culture

cell types	cell names	*p*53 states
Stem cell	**hiMSC** (human immortalized mesenchymal stem cell)	−/−
Normal cell	**BJ/hTERT** (human foreskin fibroblast hTERT immortalized cells)	+/+
Cancer cell	**H1299** human p53-defective non-small cell lung cancer cells	−/−
	H1299wt/*p*53 H1299 expressing wild type *p*53 cells	+/+

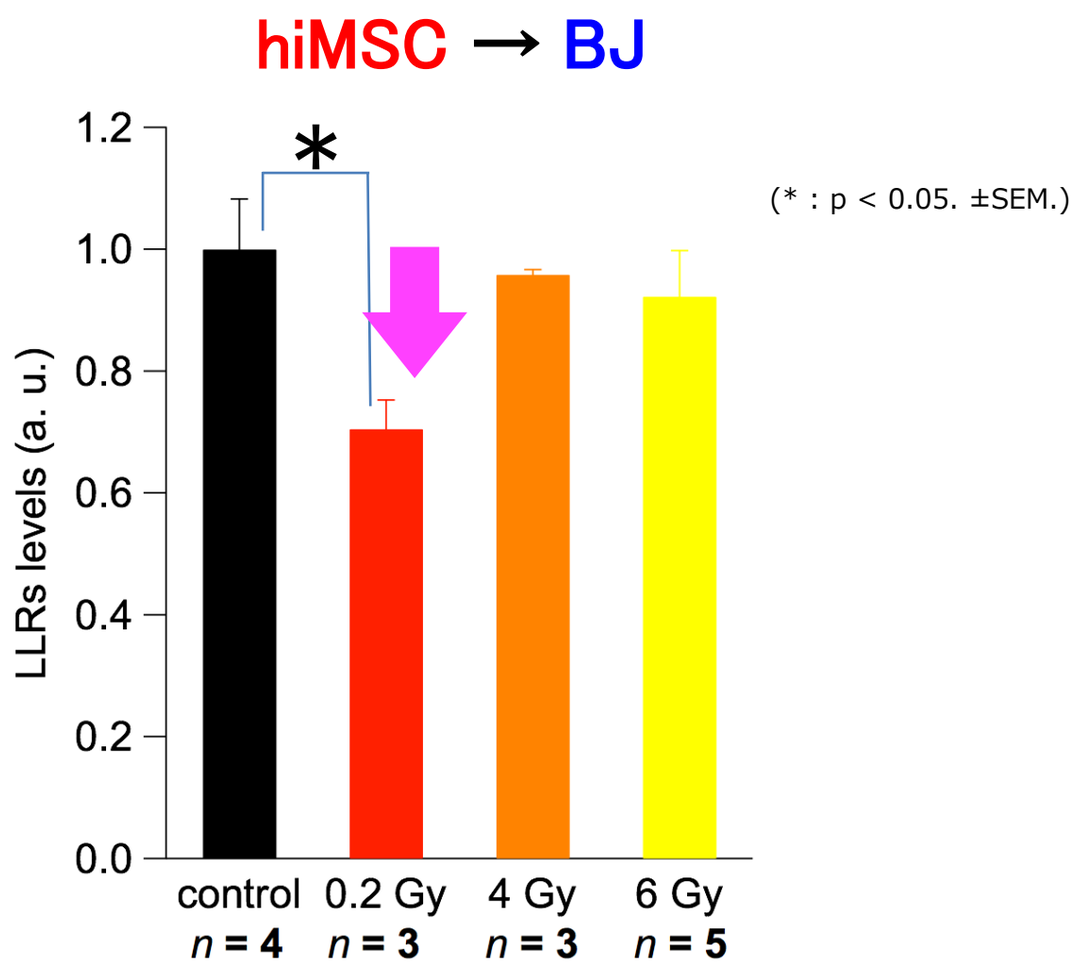
- Medium: D-MEM + 10%FBS (Fatal Bovin Serum)
- Culture Condition : 37° C, O_2 (21%), CO_2 (5.0%)

Medium transfer (MT) protocol



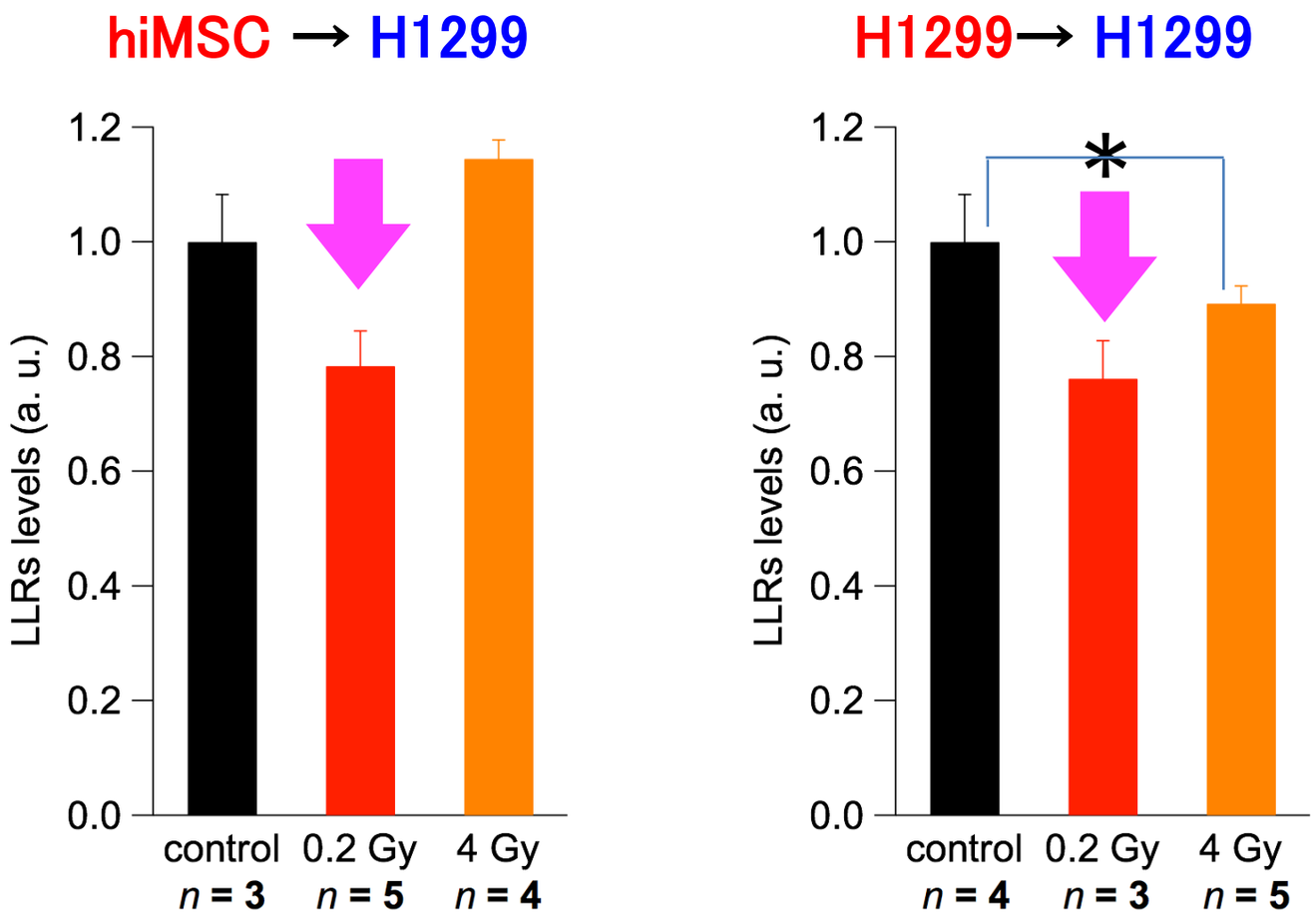
- Exosome distribution measurement
- LLRs measurement by ESR

LLRs levels in recipient BJ/hTERT cells



- Bystander factors secreted from low dose (0.2 Gy) irradiated stem cells reduce LLRs levels of normal cells but not for higher dose.
- Level down of LLRs might be related to acquisition of radiation protection ability by increasing anti-oxidant ability.

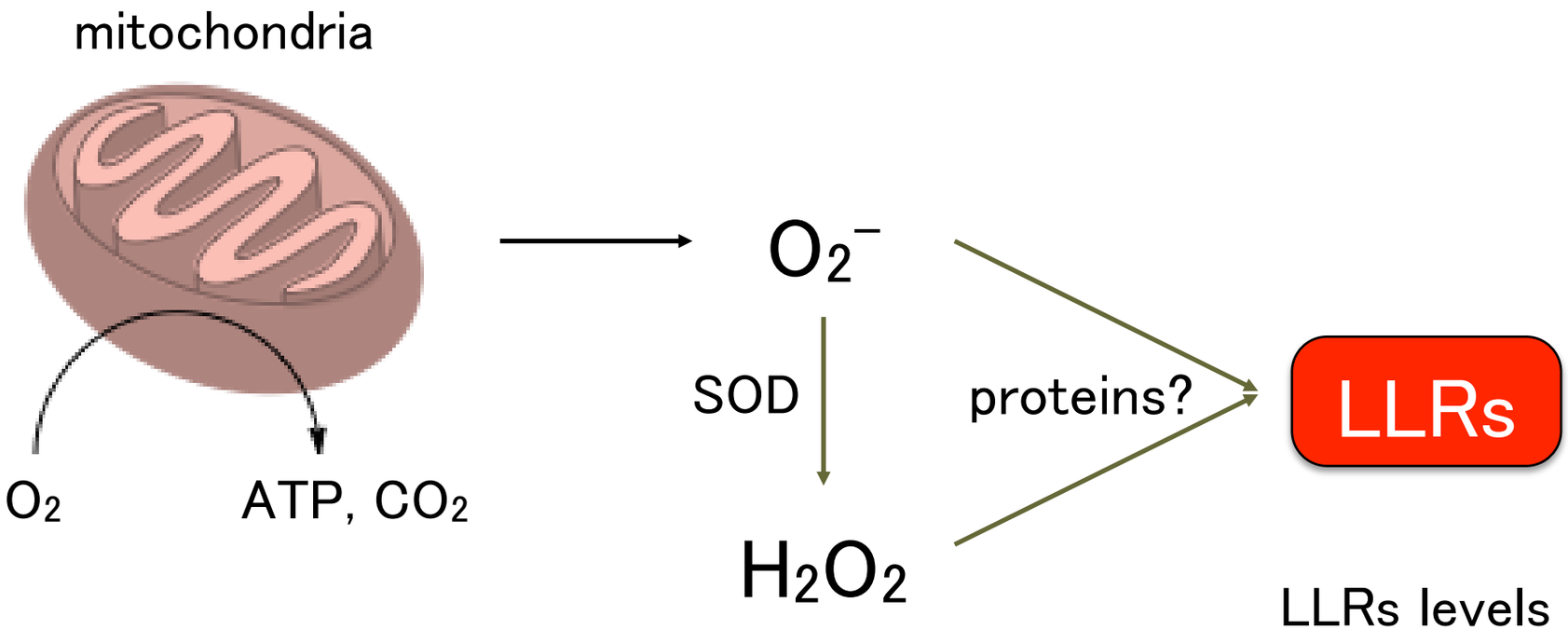
LLRs levels in recipient H1299 cells from different donor cells



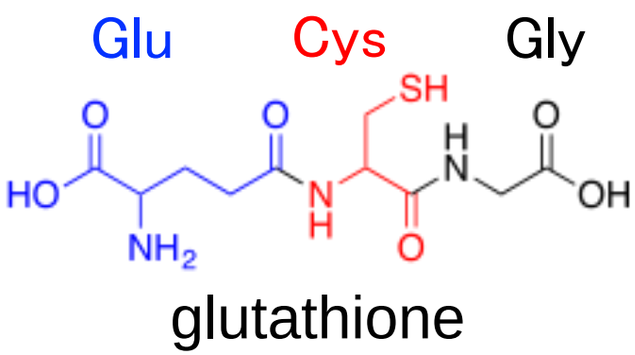
(* : $p < 0.05$. ±SEM)

- Stem and cancer cells secrete bystander factors increasing radiation protection ability to cancer cells.

LLRs levels in cells



LLRs levels reflect oxidation degree in cells.



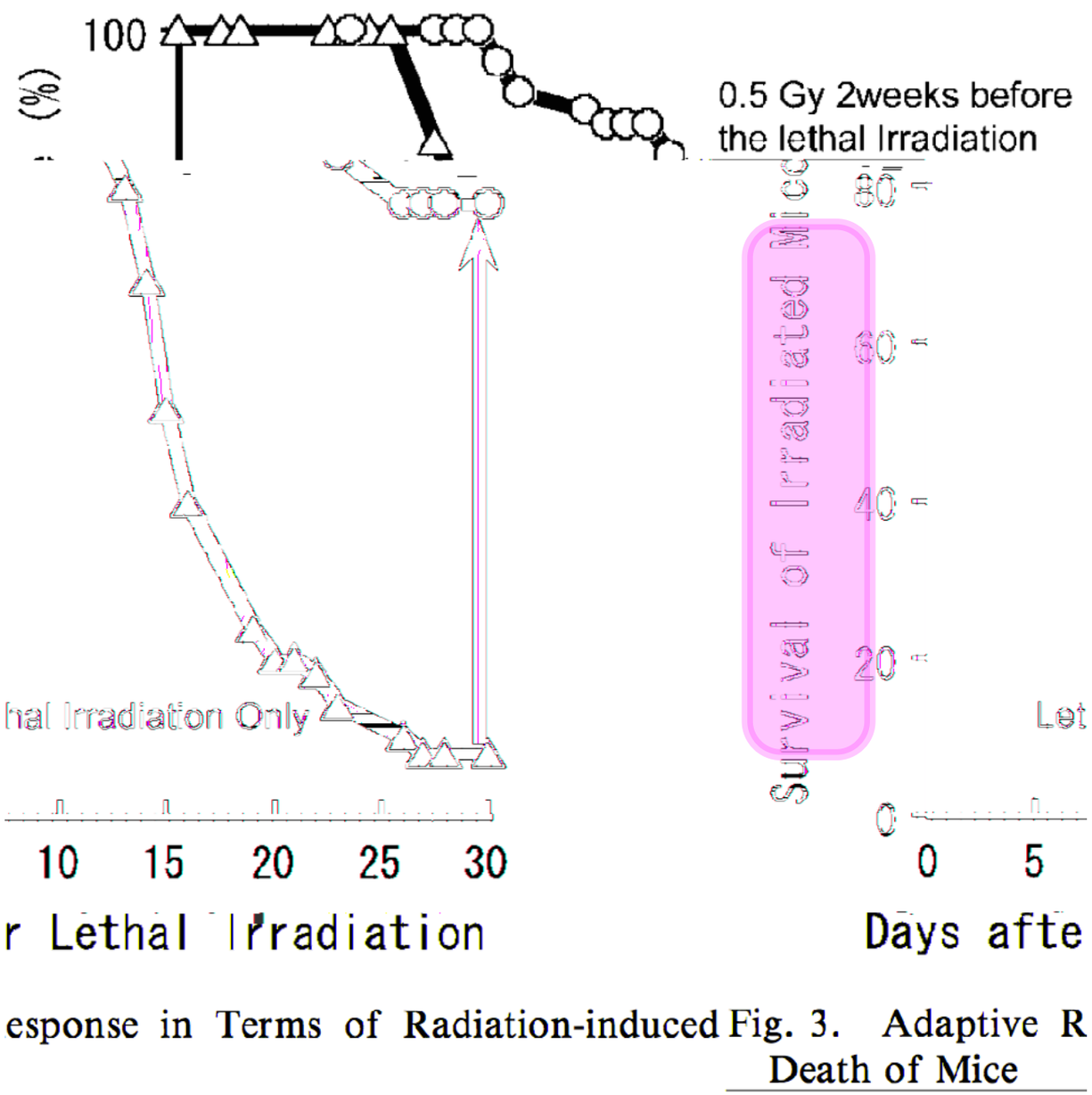
≡ GSH

glutathione

$2GSH + 2R\cdot \rightarrow 2RH + GSSG$ R·: ROS, LLRs

$GSSG \xrightarrow[GR]{} 2GSH$

Radiation Adaptive Response



esponse in Terms of Radiation-induced Fig. 3. Adaptive R
Death of Mice

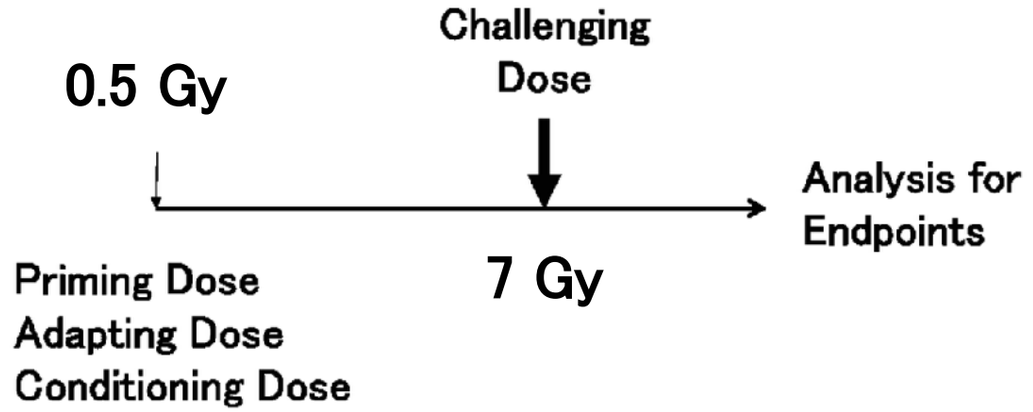


Fig. 2. A Typical Protocol to Demonstrate Adaptive Responses

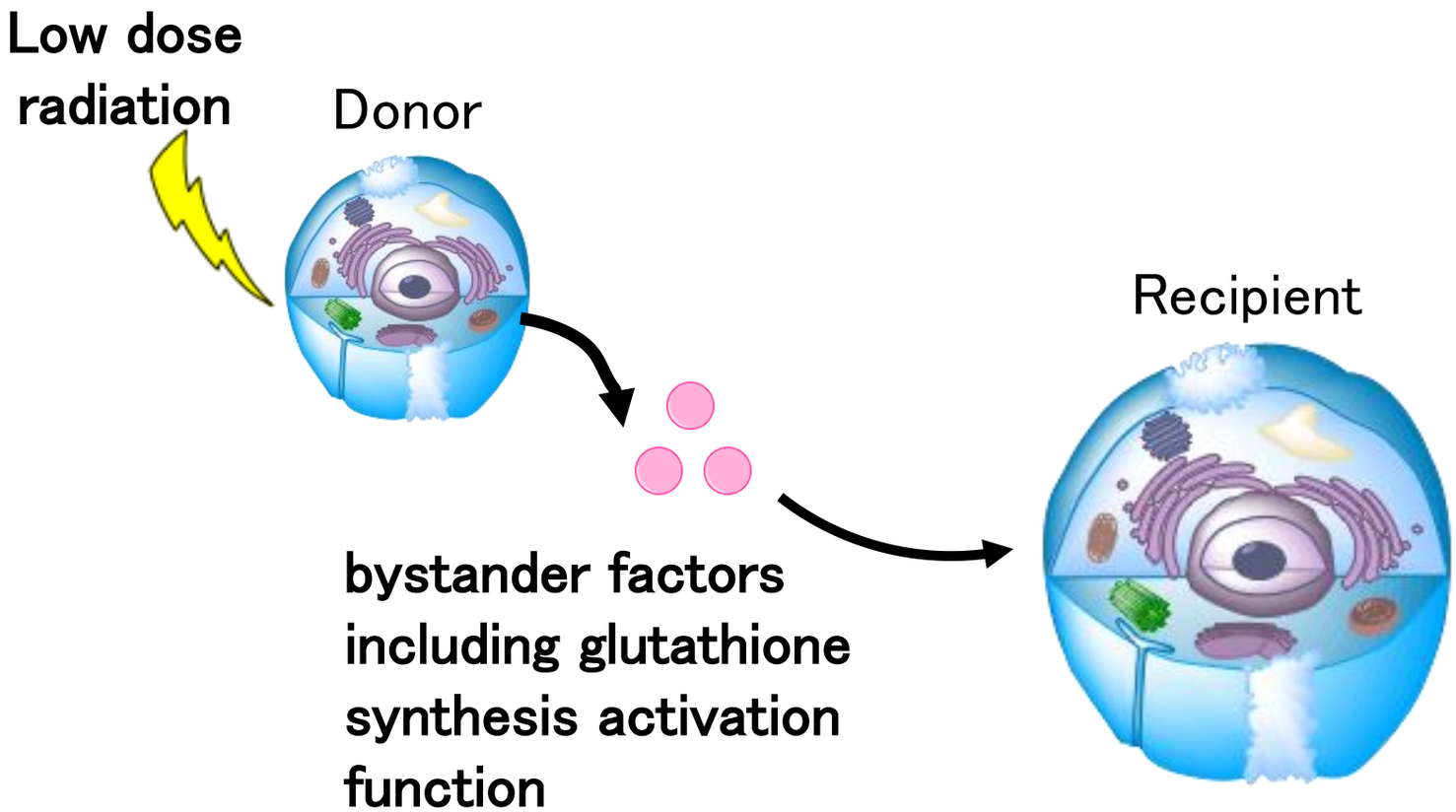
- Priming dose of 0.5 Gy by γ-ray was applied to the mice at two weeks before challenging dose irradiation of 7Gy.

- Significant improvement of survival was obtained by the priming dose (Yonezawa Effect)

Yonezawa, M., Misonoh, J. and Hosokawa, Y. *Mutat. Res.*,**358** 237–243 (1996).

酒井一夫, *Yakugaku Zasshi* **126** 827–831 (2006).

Plausible mechanisms to reduce LLRs levels in recipient cells



The bystander factors induce upregulation of GSH levels.

↓

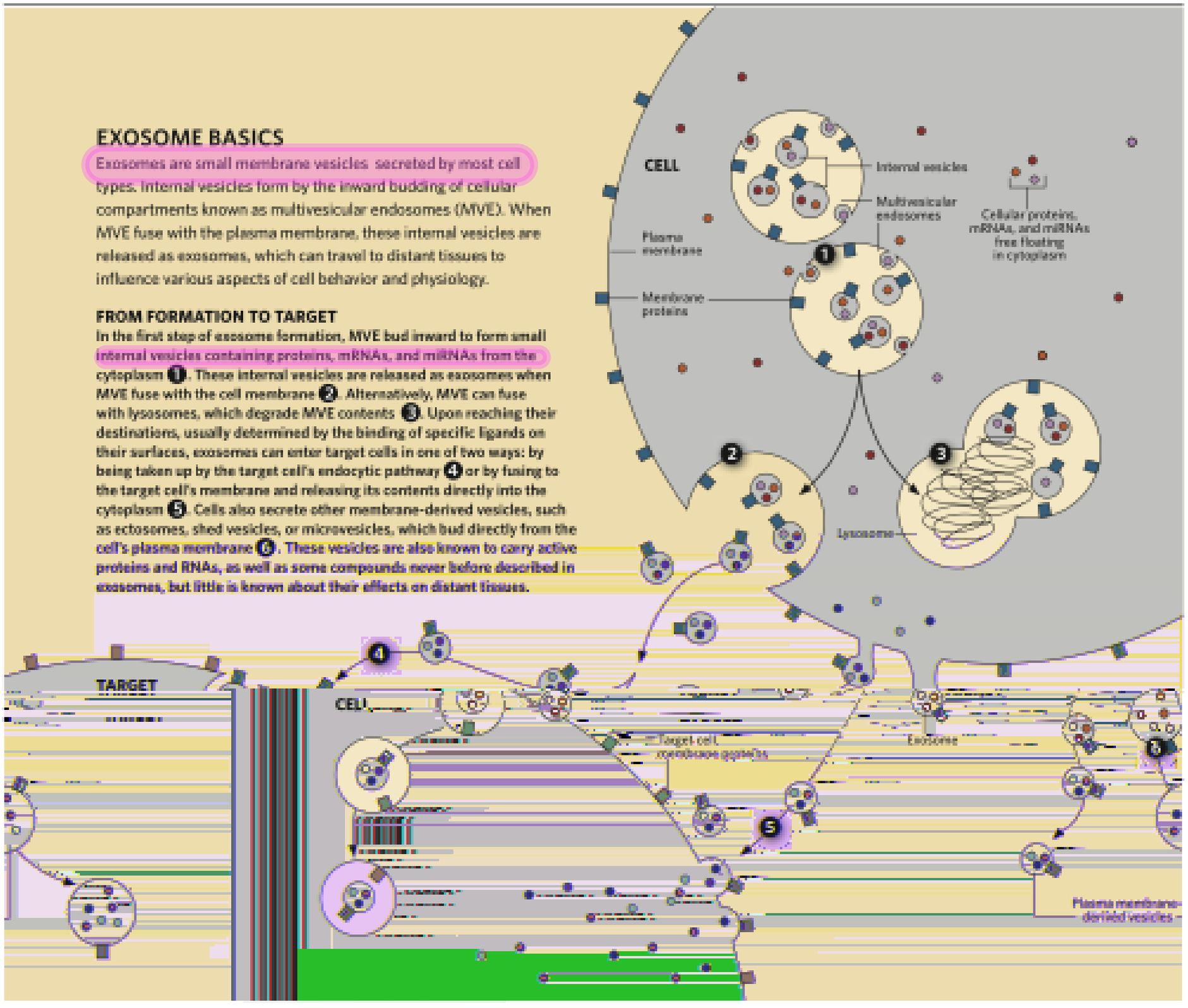
LLRs levels decrease.

Adaptive response in recipient cells for further higher dose irradiation is induced by bystander factors from donor cells irradiated in low dose.

Exosomes: secreted vesicles and intercellular communications

Clotilde Théry

F1000 Biology Reports 2011, 3:15 (doi:10.3410/B3-15)



Exosome diameter distributions

filtered medium from donor

↓

ultracentrifugation

↓ 1.1×10^6 rpm, 80 min, 4°C

−medium, + PBS(−)

↓

ultracentrifugation

↓

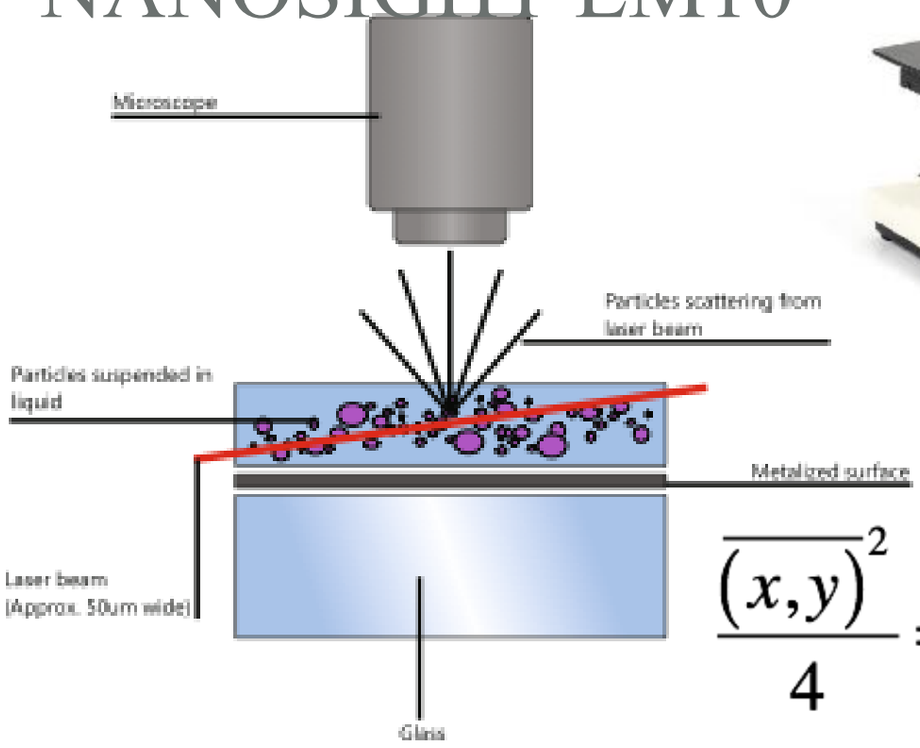
−PBS, + new PBS(−)

↓

Exosome measurement

Malvern Instruments Limited

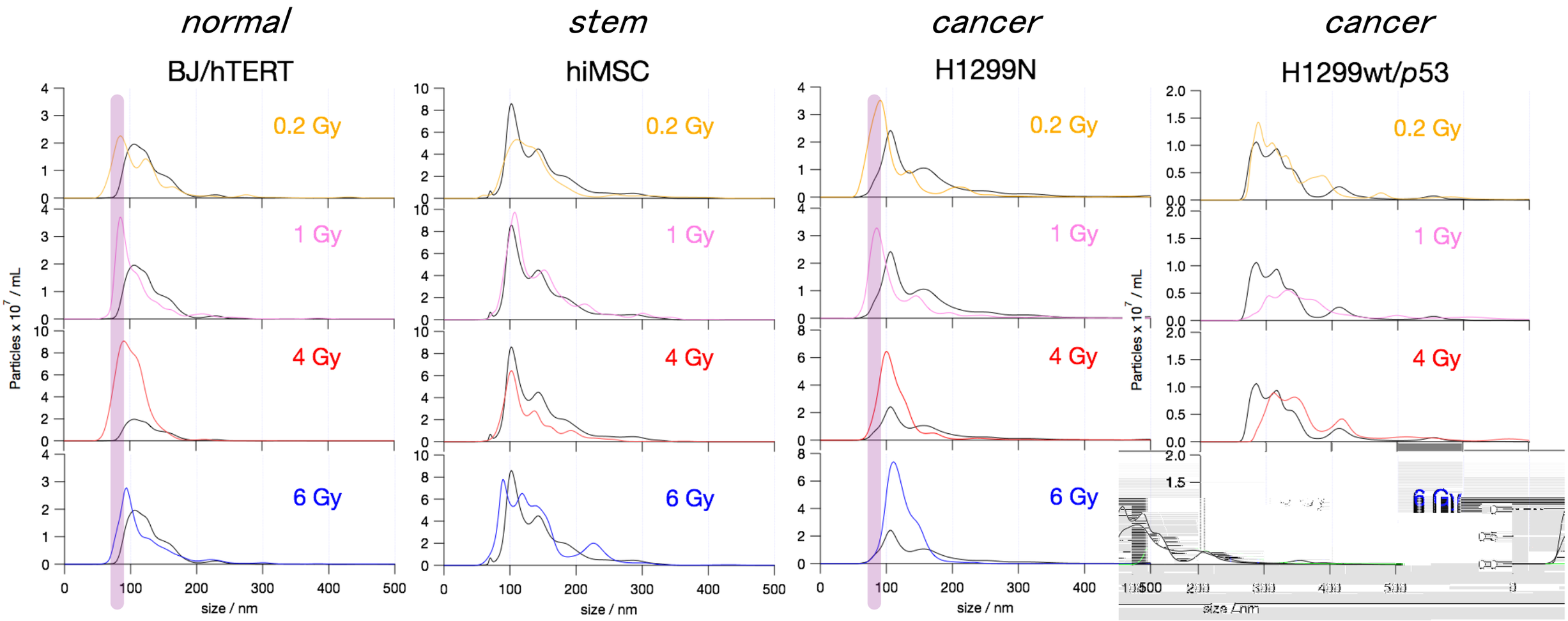
NANOSIGHT LM10



$$\frac{\overline{(x,y)}^2}{4} = Dt = \frac{Tk_B}{3\pi\eta d}$$

- Momentum of each nanoparticle can be directly analyzed by the movie of brownian motion through scattering light.
- Stokes−Einstein equation gives diameters of each particles from their momenta.

Dose dependent exosome distributions for four different cells



- Low dose (0.2 and 1 Gy) irradiation induces smaller exosome (90 nm) secretion in BJ/hTERT and H1299 cells.
- Higher dose increases secretion of exosomes especially in H1299 cells.
- p53 expression in cancer cells inhibits over secretion of exosomes.

Summary

- Donor Cells
  - Both cancer and stem cells without p53 secrete bystander factors to increase radiation protection ability in recipient normal and cancer cells by low dose irradiation.
  - Size distribution of exosomes are modulated by low dose irradiation in normal, cancer, and stem cells.
  - p53 expression in cancer cells inhibits over secretion of exosomes.
- Recipient cells
  - Both normal and cancer cells acquire higher anti-oxidant ability by receiving bystander factors from low dose irradiated stem cells (or cancer cells).

- Adaptive response in recipient cells is induced by bystander factors from donor cells irradiated in low dose.
- Exosomes induced at low dose might be related to the adaptive response.

Acknowledgment

- Authors thank to Dr. Takao Yasui (Baba Lab. in Nagoya Univ.) for helping exosome distribution measurement.

- This study is supported by
  - Public offering research for the safety of nuclear power generation, Chubu Electric Power company.
  - The joint usage / research program of the Institute of Materials and Systems for Sustainability(IMaSS), Nagoya University.