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IAEA Consultancy Meeting
Hiroshima

Biological effects of low-dose radiation on cells and their implications in cancer risk

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Research aim

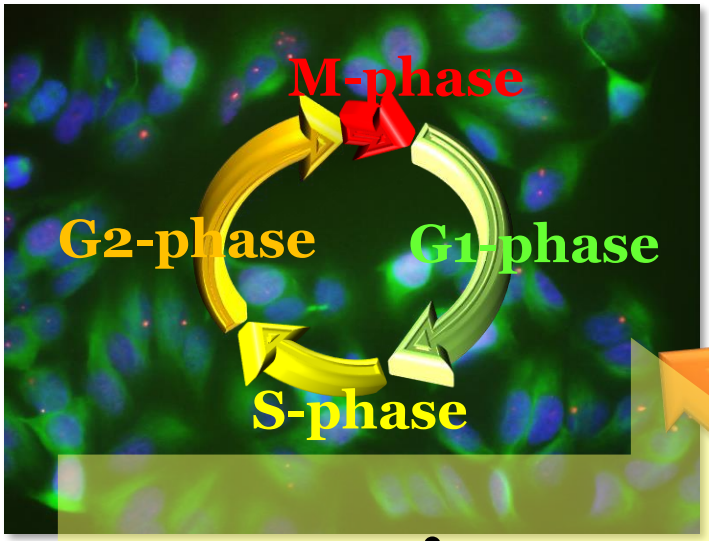
Acheiving understanding of

>The molecular basis of radiation-induced carcinogenesis
>The biological effects of low-dose radiation and their implications in cancer risk

By performing a comprehensive study of the cellular responses in different types of cells under chronic γ-irradiation

Age-related diseases and cellular responses to DNA damage

Endogenous DNA Damage
SSBs 3,000/cell/day
DSBs 0.1~50/cell/day
DNA-damaging events 20,000~
equivalent to about 3 Gy of γ-IR



Time

Age-related diseases
Aging phenotypes

with/without mutations

Various cellular dysfunctions

Aging
Stochastic→
Deterministic

Maintaining genomic stability or/and Homeostasis

Cellular responses
Respond or Not Respond

Brakes
Prevent Cell Growth
(Arrest, Death, Senescence)

DNA-damage ⇅ **DNA-repair**

Accelerators
Encourage Cell Growth, Cell Survival

Cellular responses to DNA damage and genomic mutations

Intrinsic processes
Extrinsic factors

Replication error
Replication stress

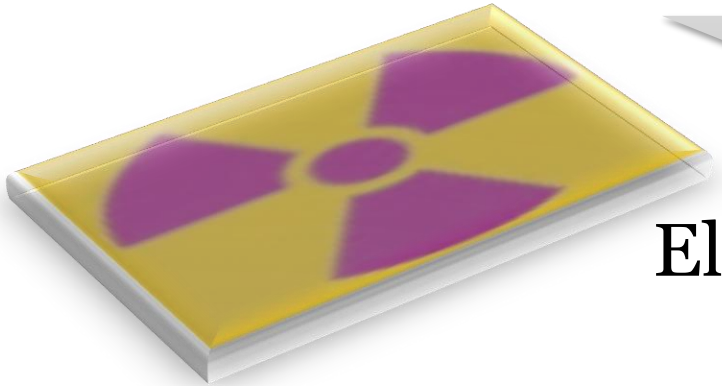
All cell types of the human body

A tissue stem cell

Mutations via cell division

--Chronic genotoxic stress-->Cancer

Tumor suppressor mechanisms



Elimination of abnormal cells

Cell senescence, Cell death,
Cell differentiation,
Cancer immunity cycle, etc.

Age-related diseases and cellular responses to DNA damage

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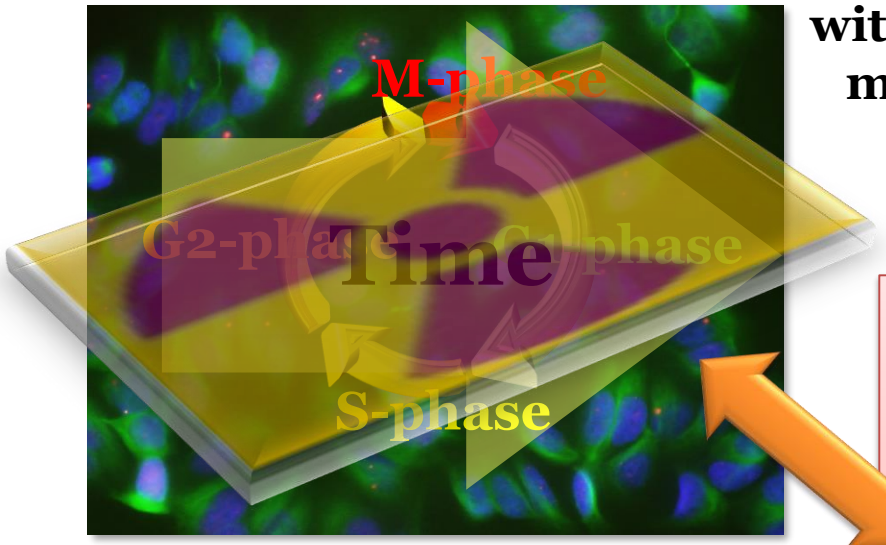

Chronic γ-irradiation
+0.001~1.0 Gy/day
SSBs +0.85~850/cell/day
DSBs +0.04~40/cell/day

Age-related diseases
Aging phenotypes

Various cellular dysfunctions

with/without mutations

Aging
Stochastic→
Deterministic

Maintaining genomic stability or/and Homeostasis

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DNA-damage ↑↓ **DNA-repair**

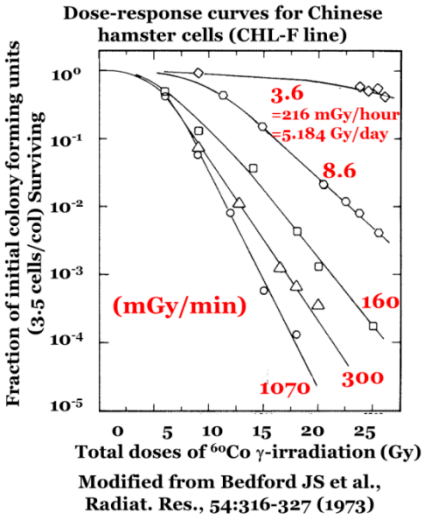
Accelerators
Encourage Cell Growth, Cell Survival

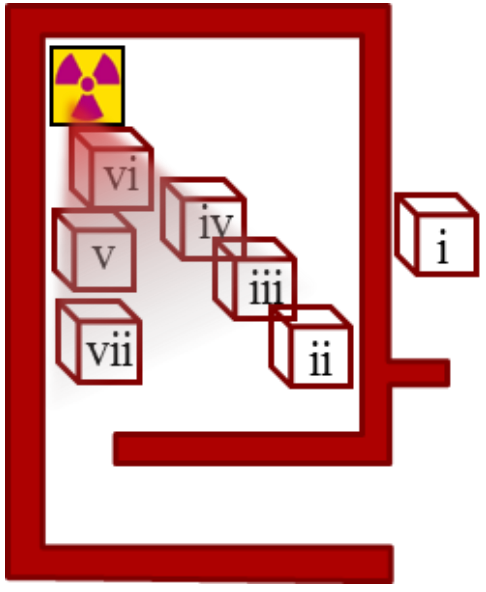
Chronic γ-irradiation over a wide range of dose-rates

Device: ^{137}Cs Low-dose-rate γ-irradiator (1.11 TBq)

i. **Background Condition (B. C.)**
ii. **0.001 Gy/day (~0.001 mGy/min)**
iii. **0.01 Gy/day (0.007 mGy/min)**
iv. **0.1 Gy/day (0.069 mGy/min)**
v. **0.5 Gy/day (0.347 mGy/min)**
vi. **1 Gy/day (0.694 mGy/min)**

Cells were cultured udner ^{137}Cs γ-rays at various dose-rates in 37°C 5 % CO_2 incubators





Chronic exposure to γ-radiation reveals significant differences in radio-sensitivity among different cell types

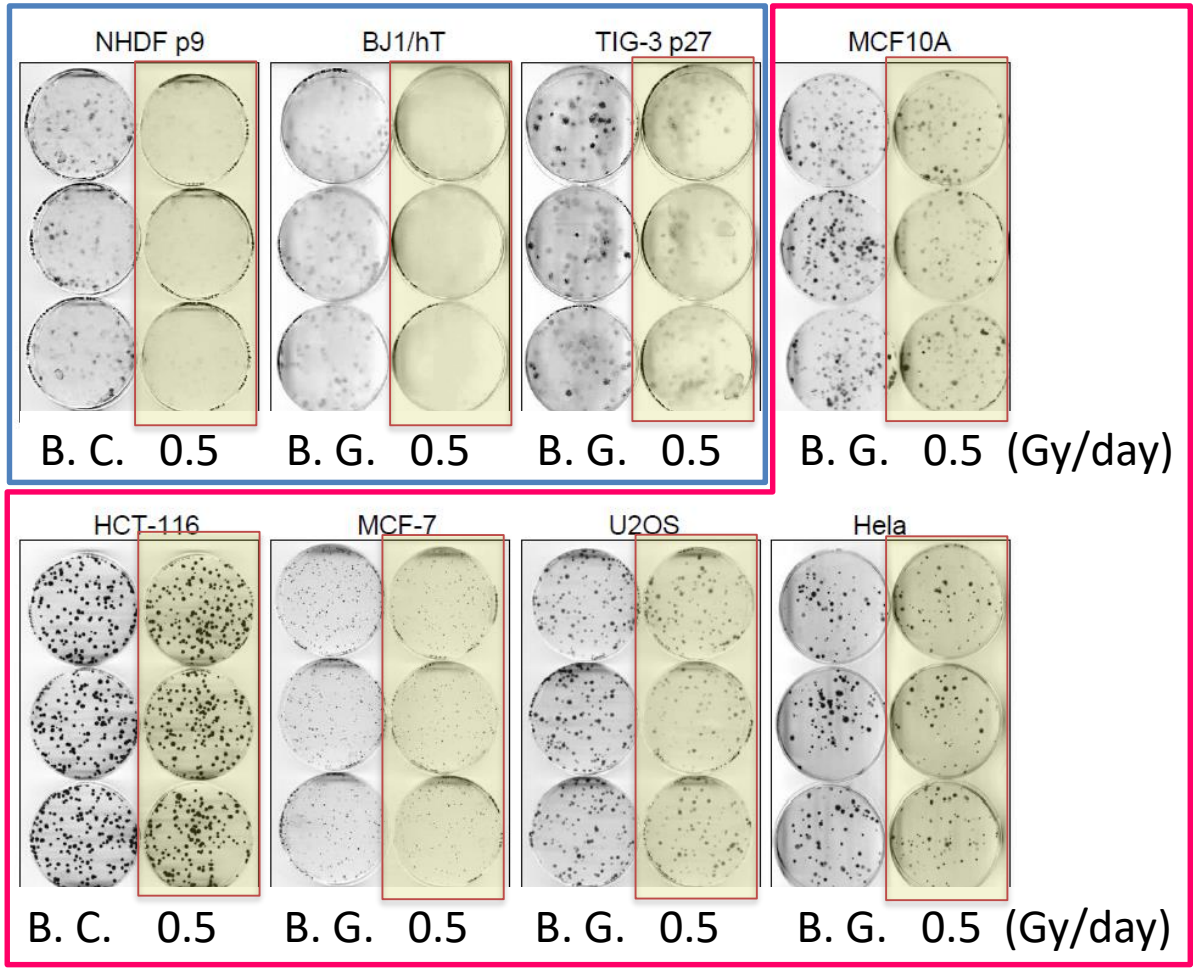
PLoS ONE 9(8): e104279. (2014)

Chronic γ-irradiation (0.5 Gy/day)

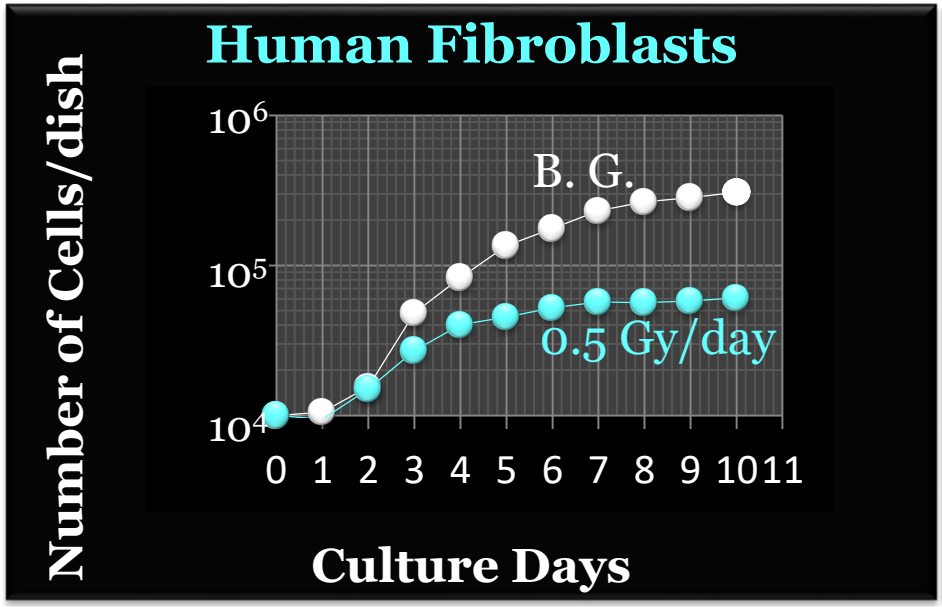
Low dose-rate γ-irradiator

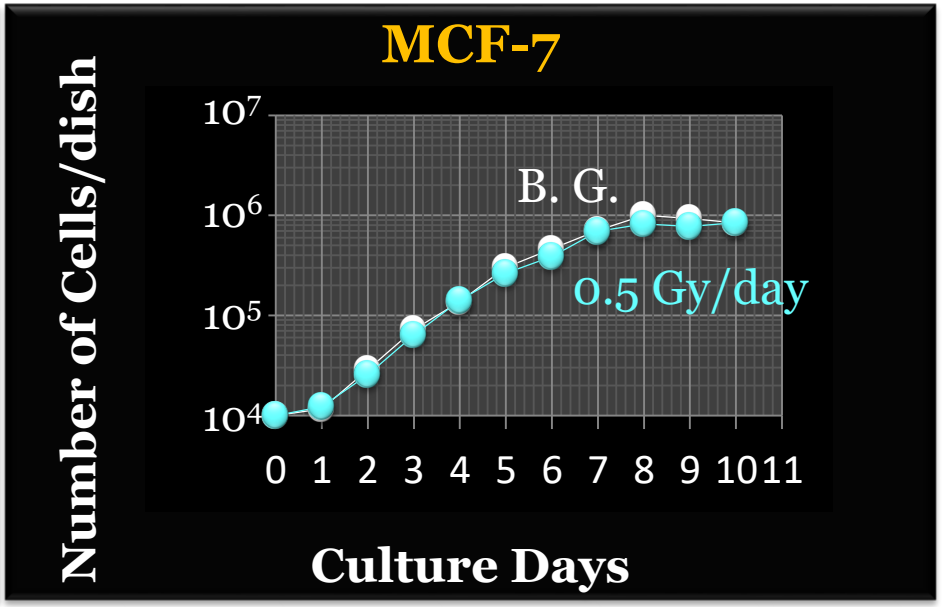
Fibroblasts

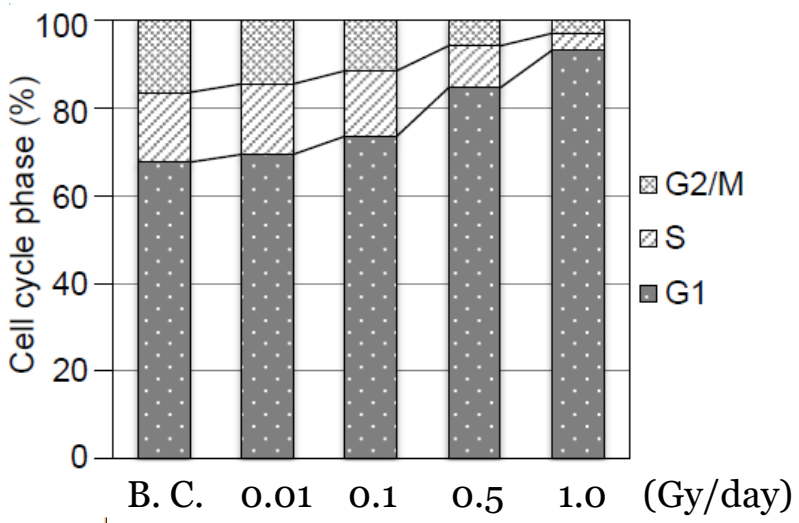
Other cell lines

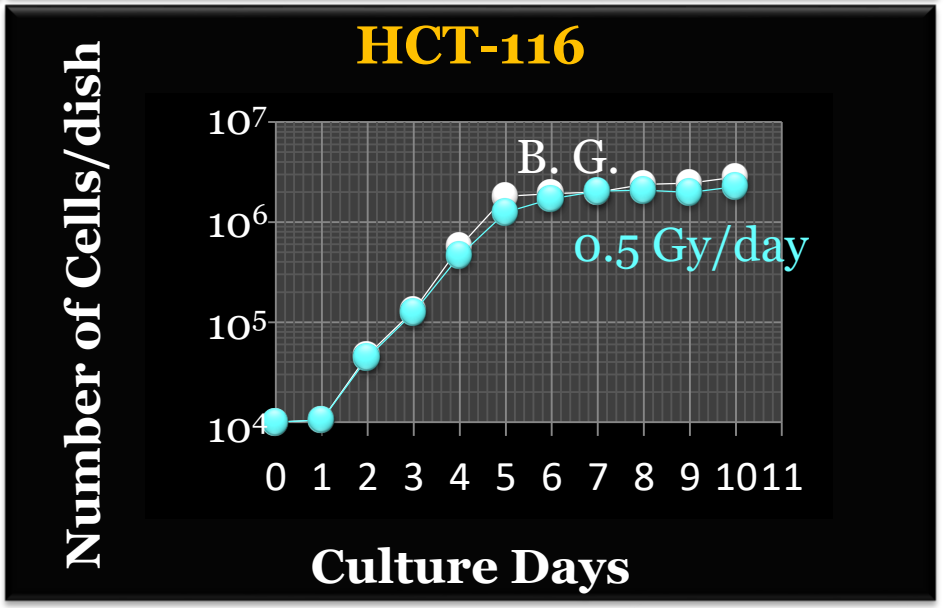


Cellular proliferation during γ-irradiation (at 0.5 Gy/day)









PLoS ONE 9(8): e104279. (2014)

Comparison of colony forming abilities of different types of cells : acute versus chronic γ-irradiation (Total dose : 5 Gy)

Cell line	Surviving fraction (Mean ±SD)	
	Acute γ-irradiation (dose rate of 1.0 Gy/min for 5 min, total dose; 5 Gy)	Chronic γ-irradiation (dose rate of 0.347 mGy/min for 10 days, total dose; 5 Gy)
NHDF p9	0.026 ±0.003 2.6%	0.069 ±0.015 6.9%
BJ1/hT	0.033 ±0.005 3.3%	0.041 ±0.013 4.1%
TIG-3 p27	0.037 ±0.026 3.7%	0.119 ±0.038 11.9%
MCF10A	0.094 ±0.006 9.4%	0.643 ±0.055 64.3%
HCT-116	0.039 ±0.009 3.9%	0.942 ±0.015 94.2%
MCF-7	0.037 ±0.005 3.7%	0.914 ±0.027 91.4%
U2OS	0.012 ±0.003 1.2%	0.933 ±0.010 93.3%
Hela	0.033 ±0.003 3.3%	0.924 ±0.068 92.4%

Fibroblasts

Other cell lines

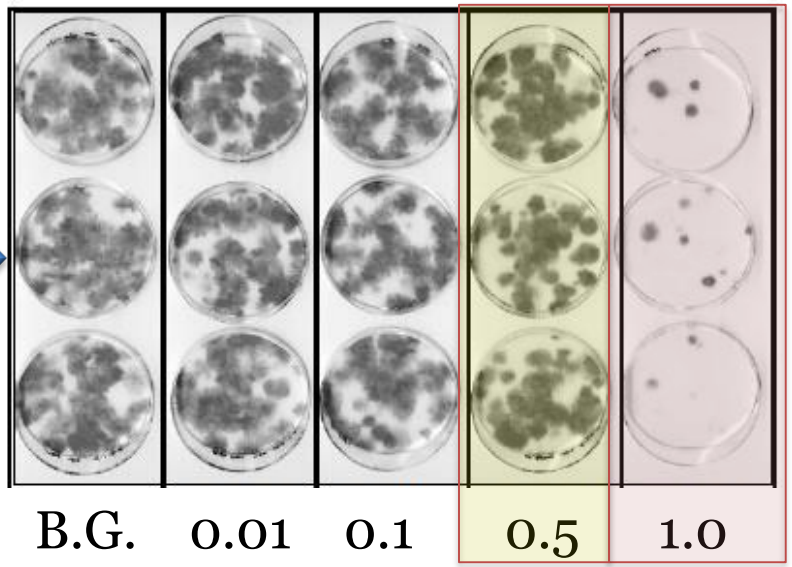
PLoS ONE 9(8): e104279. (2014)

Fibroblasts are highly responsive to chronic genotoxic stress

Fibroblasts undergo senescence when irradiated at a dose-rate of 1.0 Gy/day

Seeded at 100 cells/dish
Cultured for 10 days
at B. C., 0.01, 0.1, 0.5, 1.0 (Gy/day)

Cultured
for an additional 10 days
at B. C. following Ch-IR



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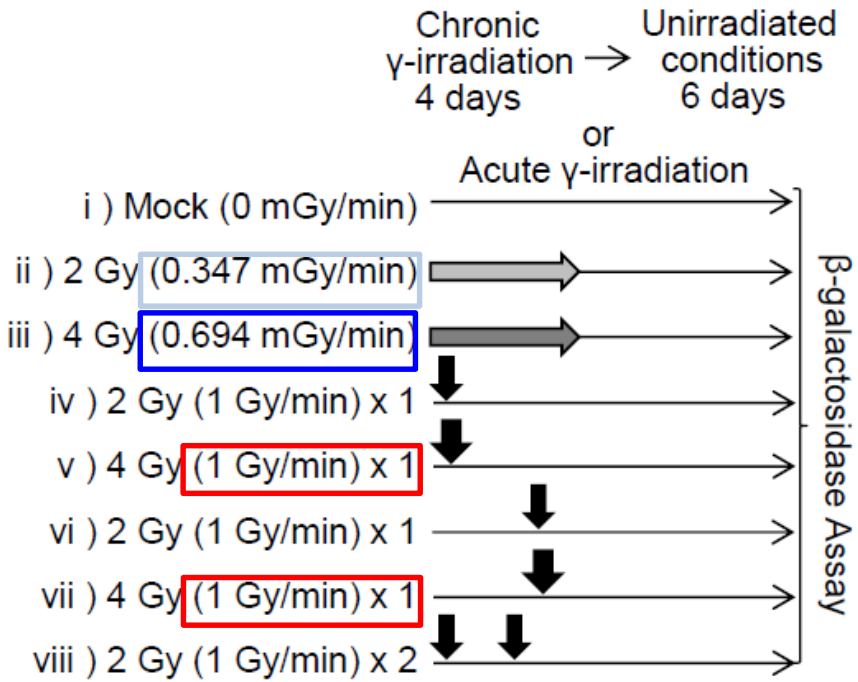

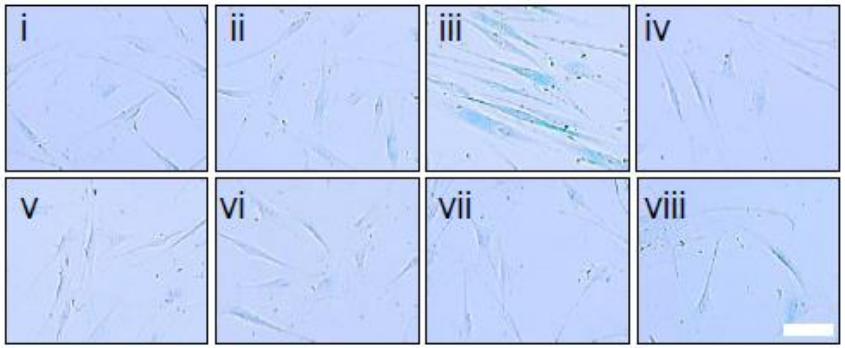

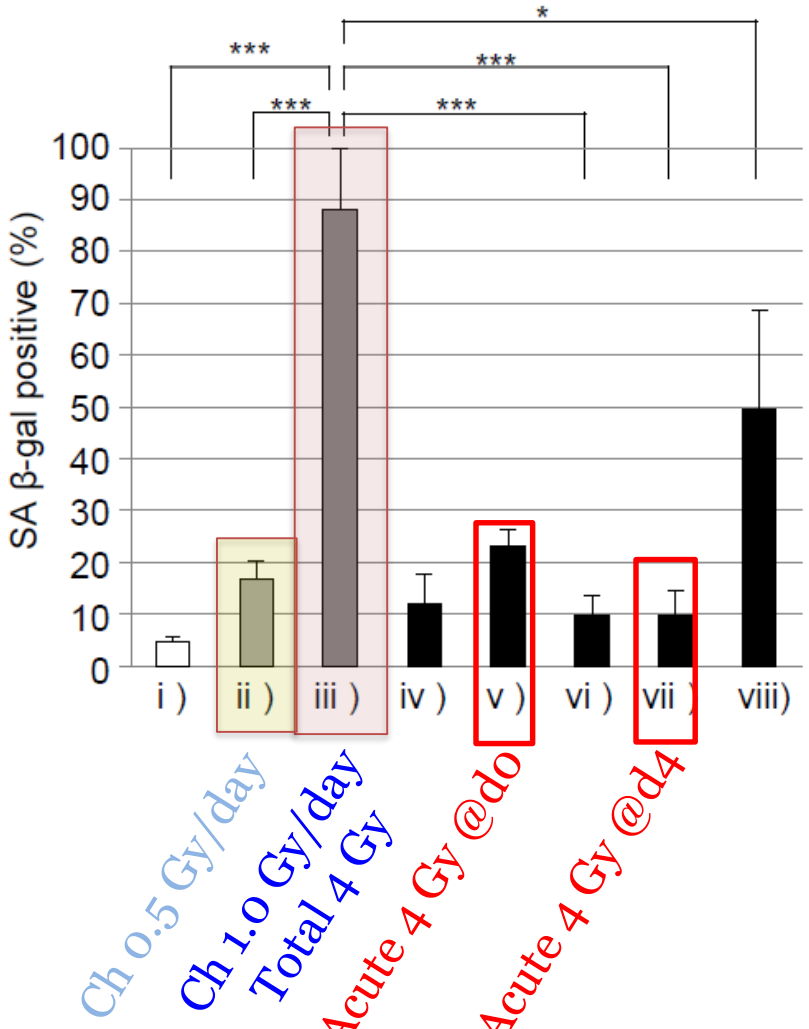

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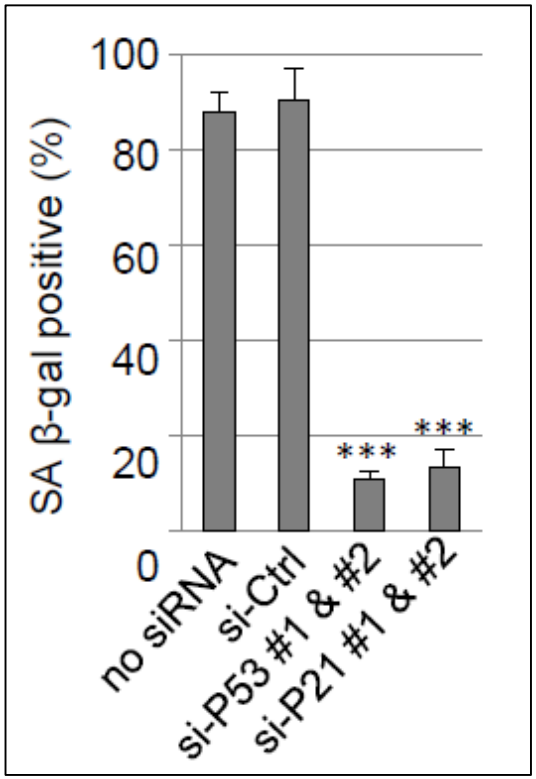
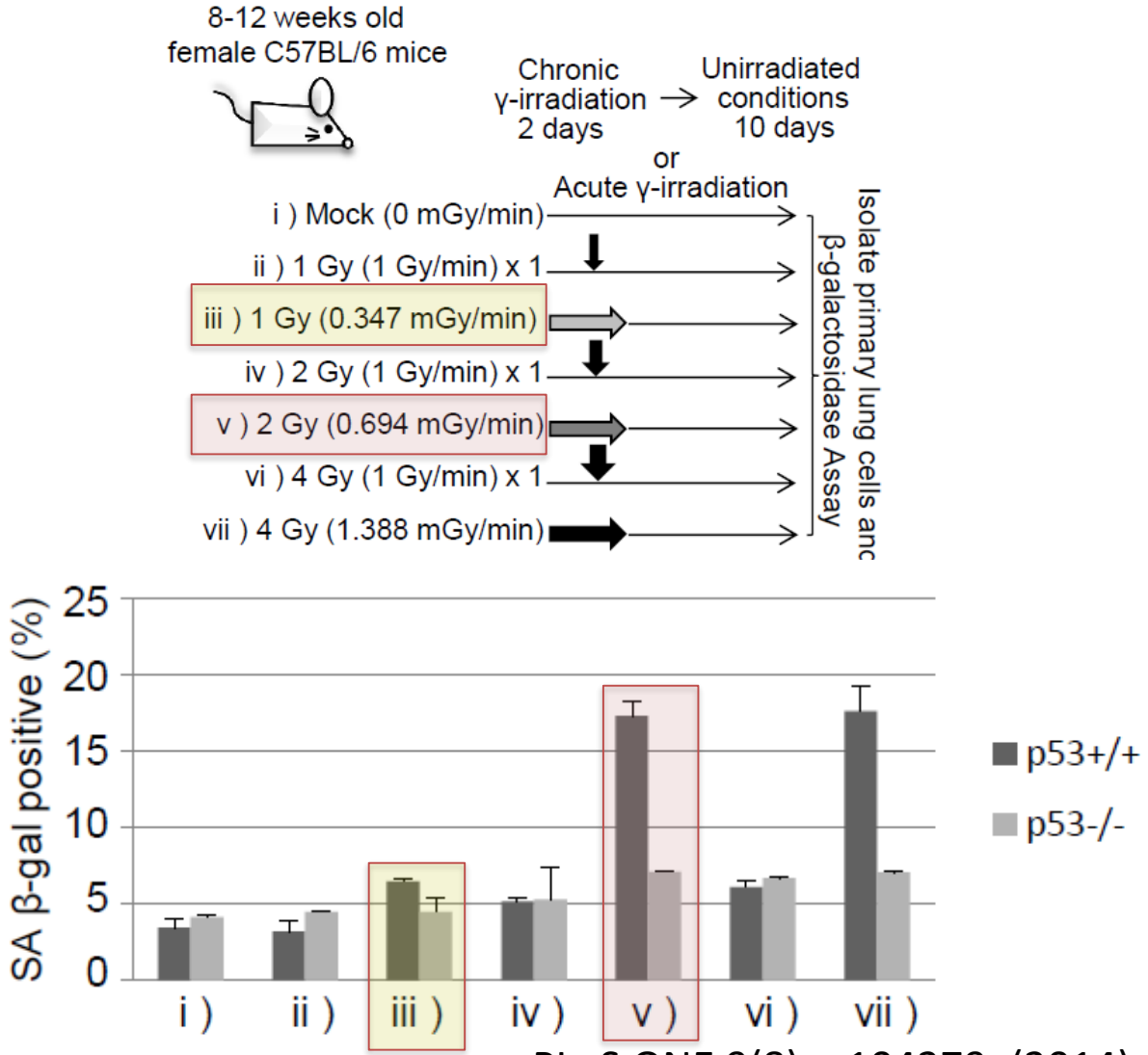
The ATM/TP53/P21 pathway is essential for chronic γ-IR-induced cellular senescence





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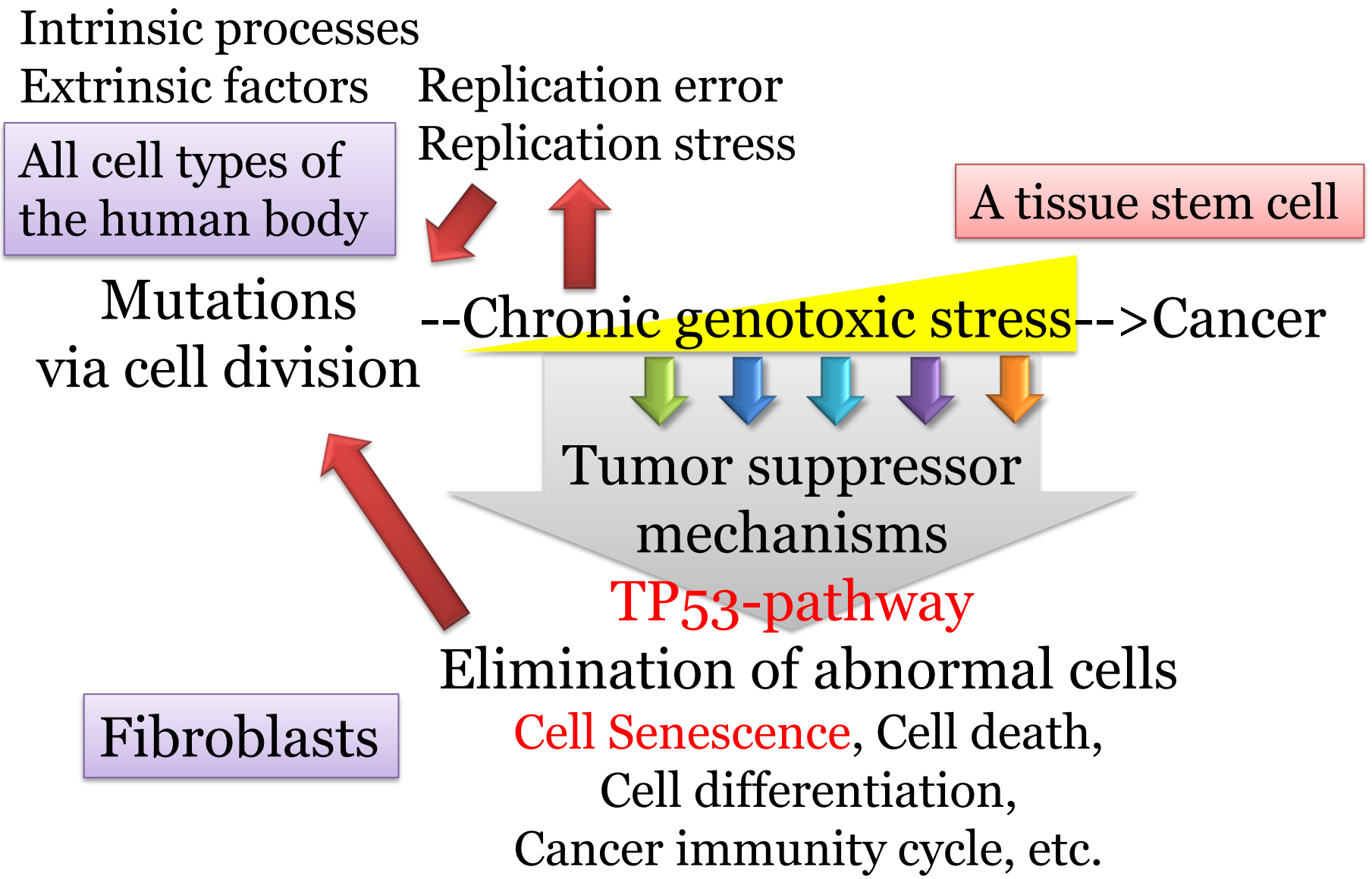
Summary (I)

- **Different cell types exhibit different response sensitivities to chronic γ-irradiation.**
- **Proliferating fibroblasts are particularly sensitive to chronic γ-irradiation.**
- **A dose-rate threshold exists for the induction of senescence-like growth arrest (between 0.5 and 1.0 Gy/day).**
- **The ATM/TP53/P21 pathway plays a crucial role in response to chronic γ-irradiation for the maintenance of genomic integrity in fibroblasts.**

Cellular responses to DNA damage and genomic mutations



On-going research

Detection/Quantification of molecular changes in fibroblasts under chronic γ-irradiation

>RNA sequencing by Next generation sequencer

→RNA expression profiles

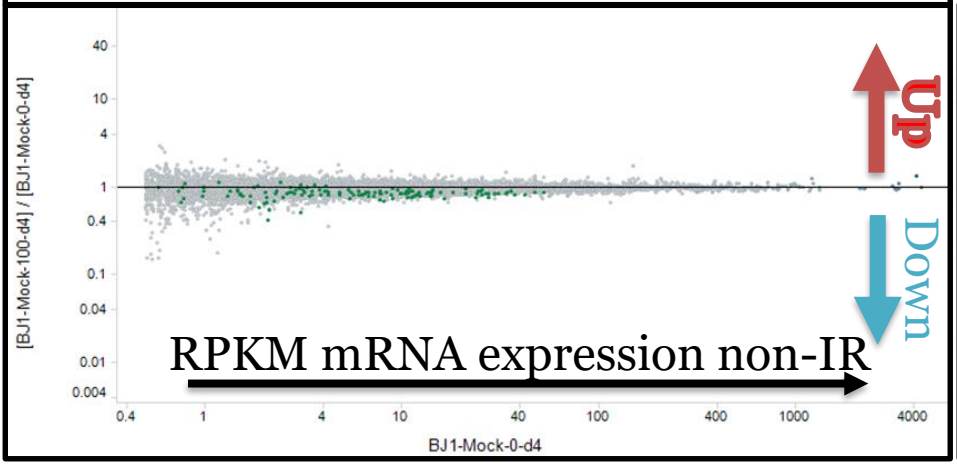
→Bioinformatics

Next generation sequencing technologies Transcriptome analysis of fibroblasts following chronic γ-irradiation

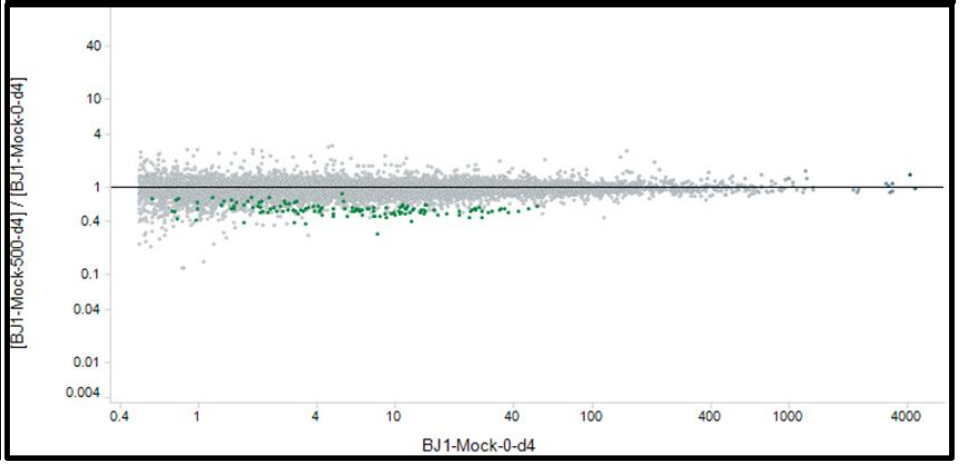
<u>Transcriptome analysis</u>
Human fibroblasts
non-IR, 0.01, 0.1, 0.5, 1.0 Gy/day, for 4 days
→Isolation of mRNA → RNA-seq by NGS

RNA-Seq (RPKM ratio, 4 days Ch-IR)

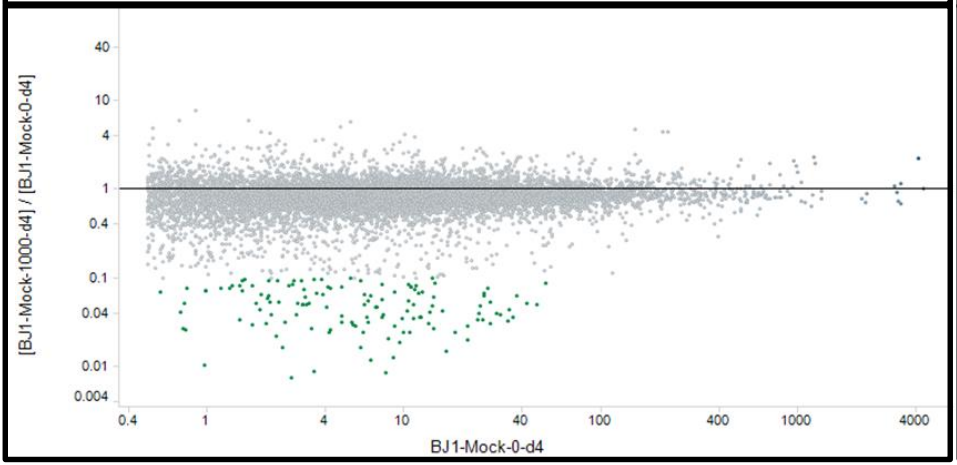
0.1 Gy/day vs non-IR (Growth suppression)



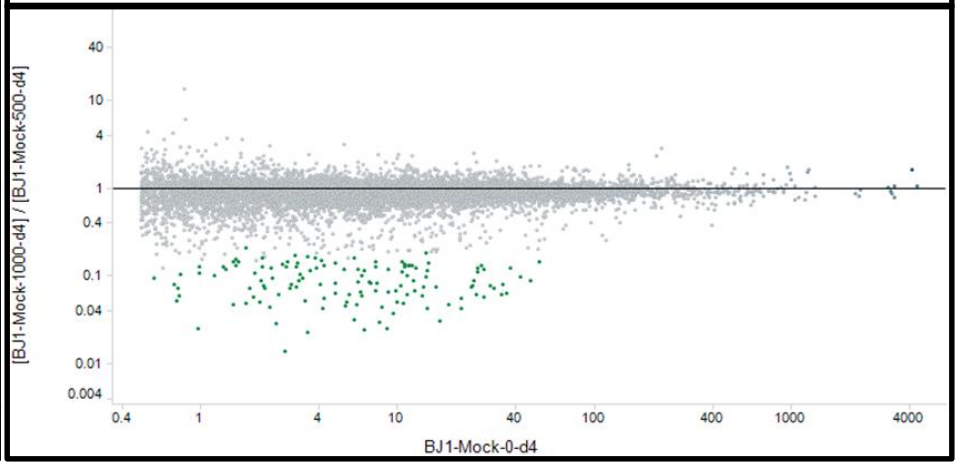
0.5 Gy/day vs non-IR (G1-arrest)



1.0 Gy/day vs non-IR (Senescence)



1.0 Gy/day vs 0.5 Gy/day (Senescence vs G1-arrest)



On-going research

Detection/Quantification of molecular changes in fibroblasts under chronic γ-irradiation

>RNA sequencing by Next Generation Sequencer

→RNA expression profiles

→Bioinformatics

Identification of responsible genes and pathways for cellular responses and cell-fate decisions

>siRNA library screening by High-throughput Imaging Systems

→Cellular phenotype

→Systems biology

High-Throughput siRNA Screening Identification of the genes responsible for the cellular responses to γ-irradiation

siRNA library for 21,584 genes (96well-plate 258-plates)

siRNA-x	siRNA1-x + Ctrl-si
siRNA-x + TP53-si	siRNA-x + P21-si

x 96 siRNA

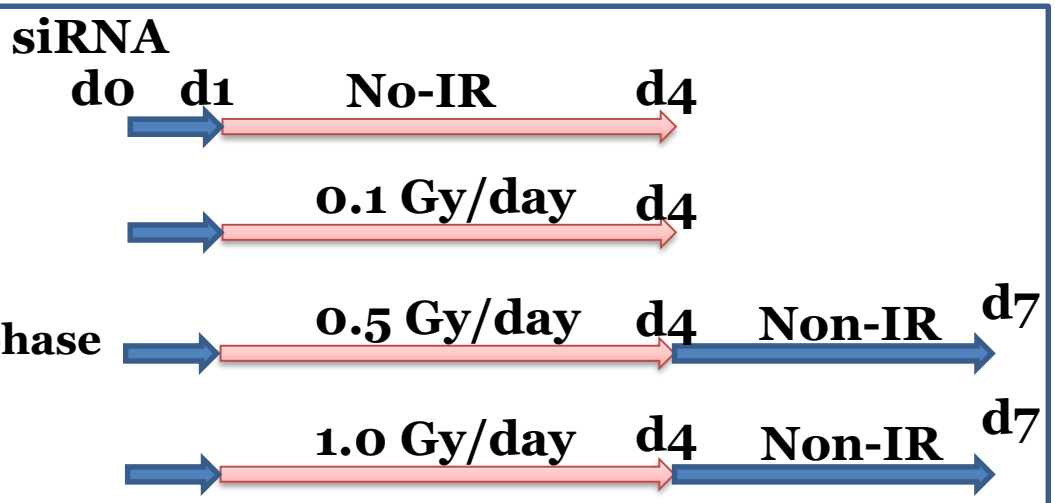
1 gene/4-wells

1) Un-irradiated condition

2) (Partial) Growth suppression

3) (Reversible) Growth arrest at G1-phase

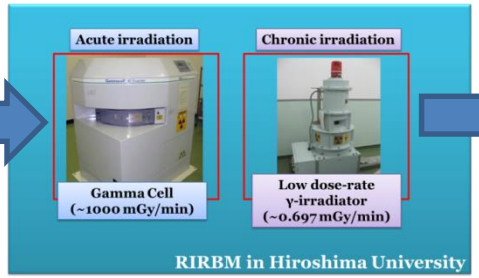
4) Senescence-like growth arrest



High-Throughput siRNA Screening

siRNA library for 21,584 genes
（96well-plate 258-plates）

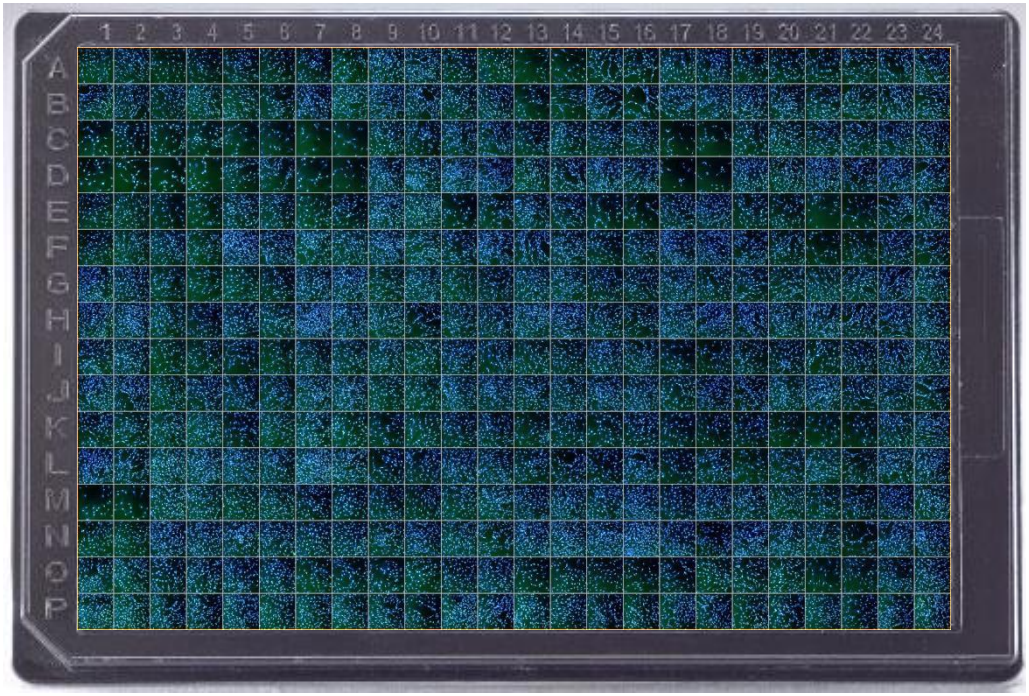
384-well plate



Nuclear DNA: Hoechst
Anti-53BP1: Alexa 488
→DNA damage, Repair
Anti-Ki67: Alexa 555
→Proliferation

High-Throughput Imaging Systems

Opera phenix™



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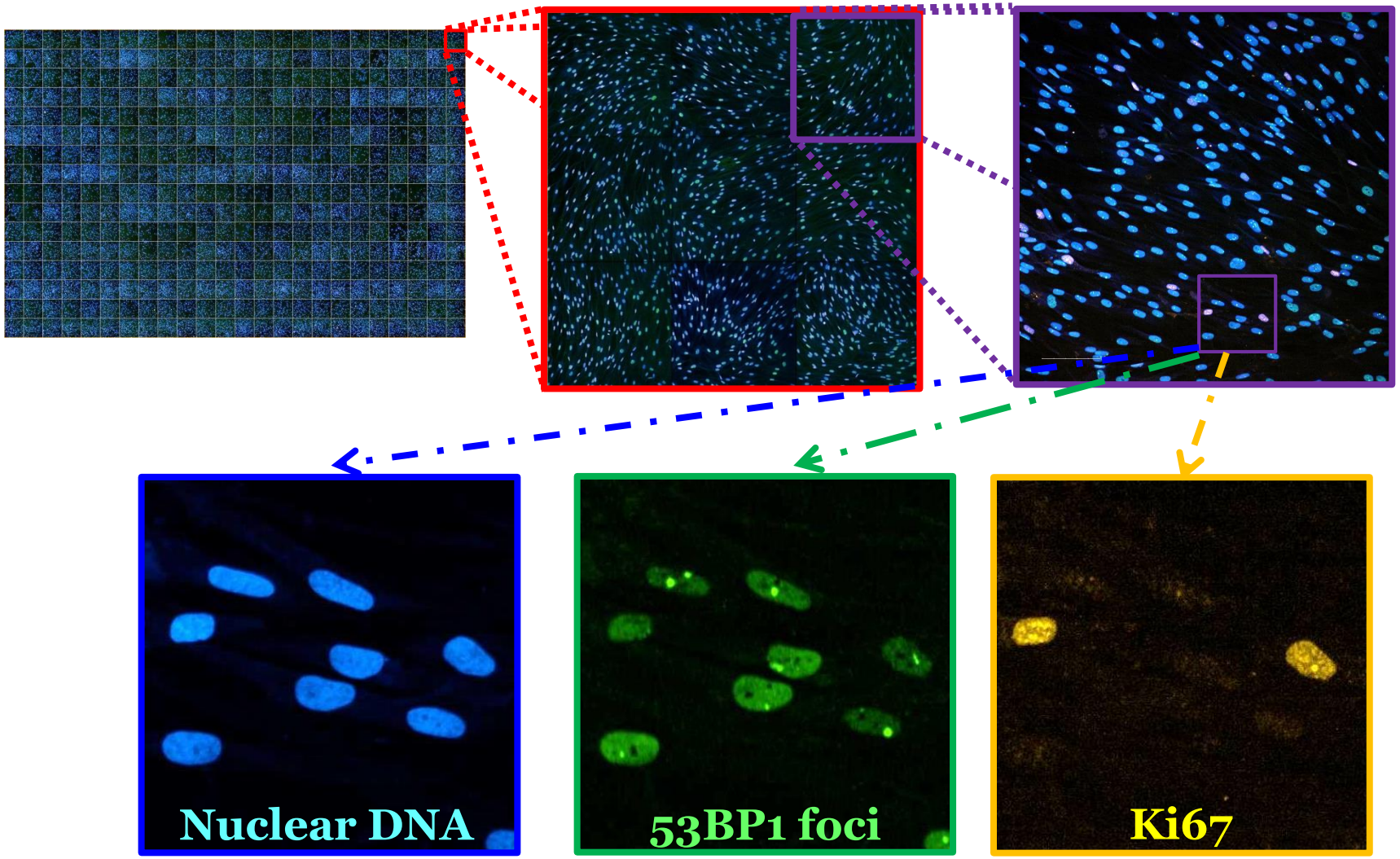
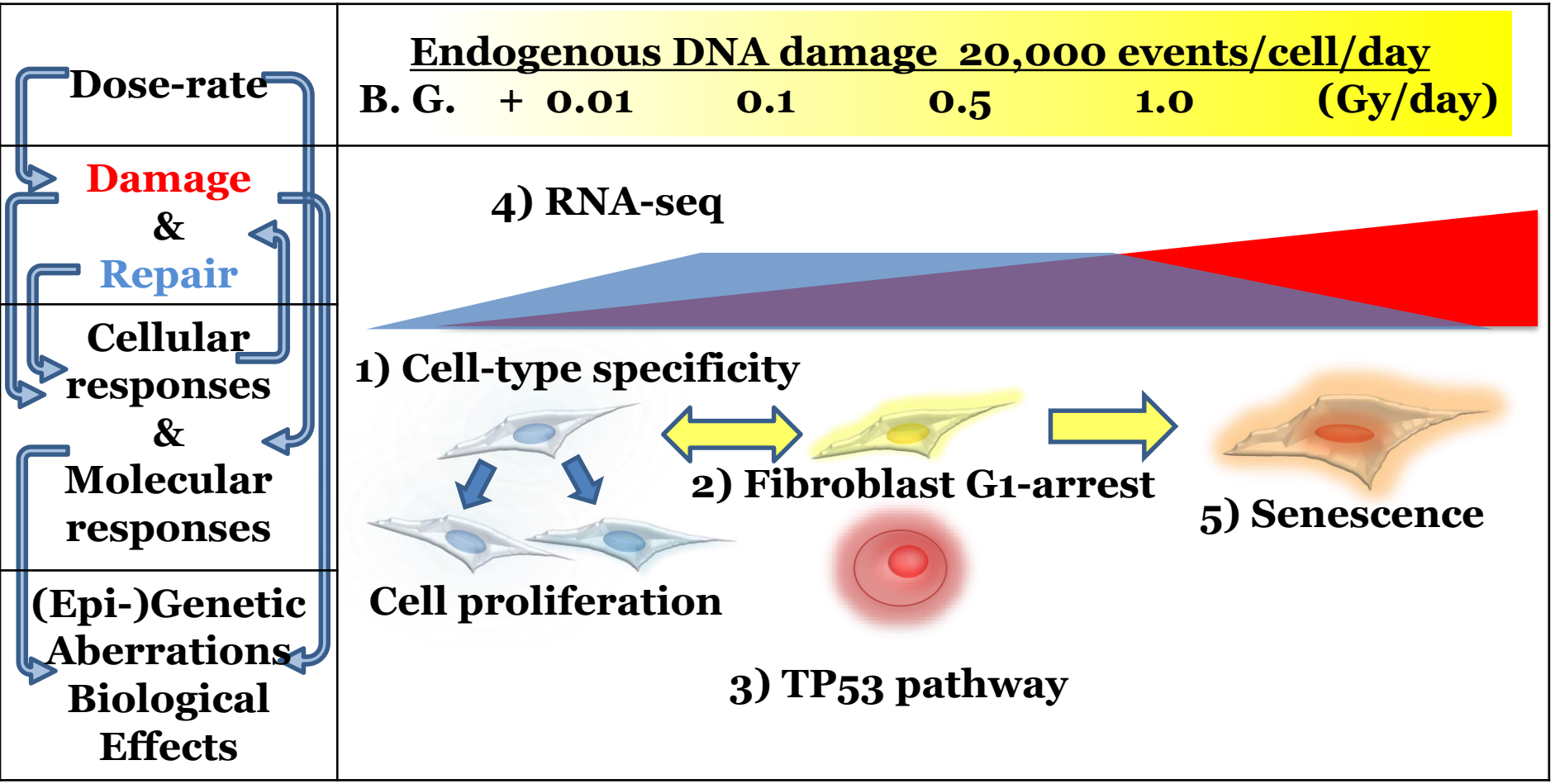
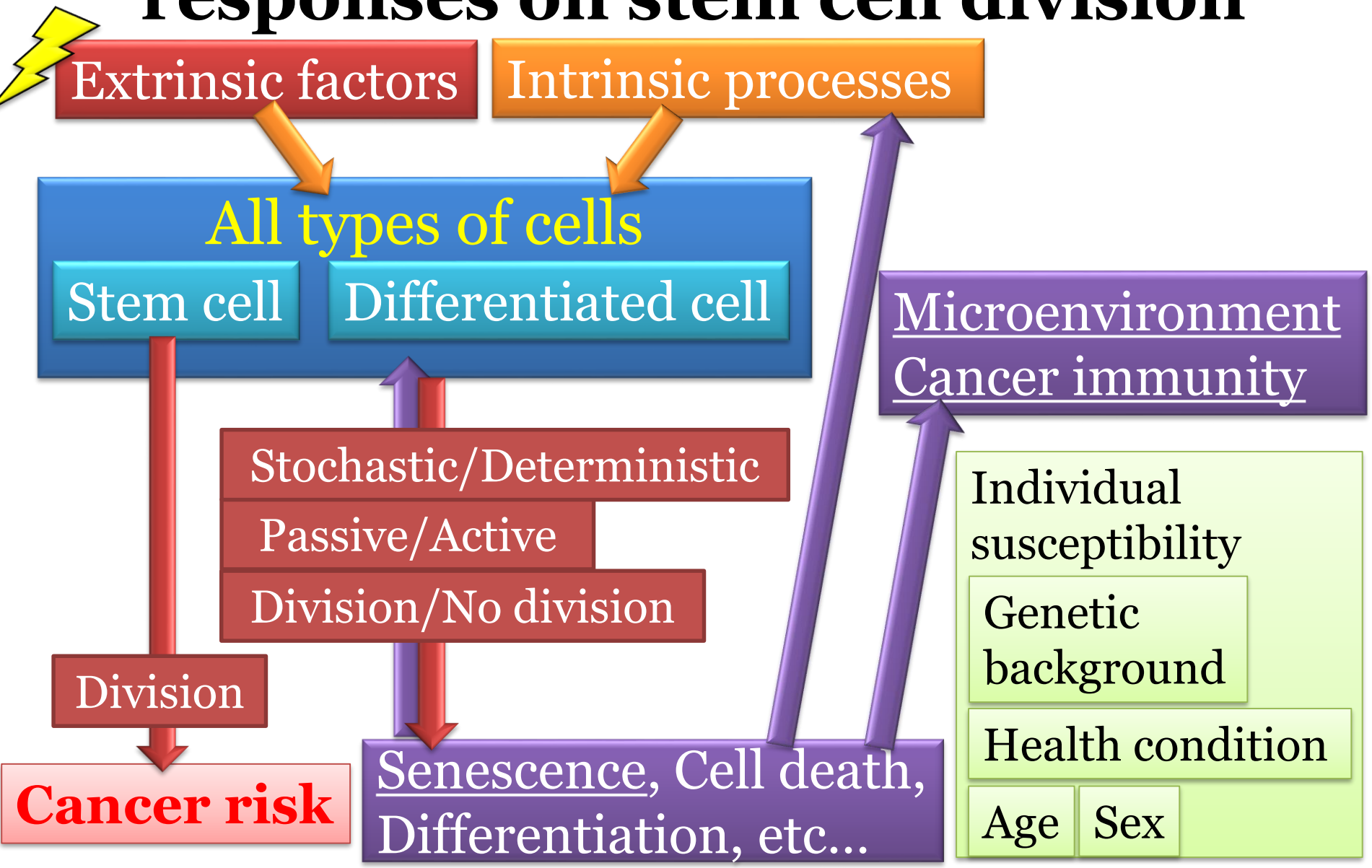


Summary (II)



High-density time- and dose-rate-dependent measurements of gene expression profiles and cellular responses during chronic γ-irradiation

The complex effects of cellular responses on stem cell division



The Research Perspective

Comprehensive studies of cell-type-, dose-rate- and time- dependent cellular responses and subsequent cell-fate decisions during chronic γ-irradiation (especially in vivo, if it is possible) may help us to better understand the relevant targets for the biological effects of low-dose radiation.

The results from these studies may provide us with more insights into individual susceptibility to low-dose radiation and with the molecular basis for epidemiological studies on radiation risks of low-dose radiation exposure.