Mechanisms Underlying Low Dose $\gamma$-Ray-Induced Adaptive Responses

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Plan:

- Evidence for adaptive responses induced by low dose/low dose-rate $^{137}\text{Cs-}\gamma$ rays in $G_0/G_1$ normal human fibroblasts

- Evidence for mediating mechanisms

- Discussion:
  Biological factors modify the cellular response to energy deposition events
The frequency of human exposure to low level ionizing radiation is on the increase.

Thus, the evaluation of risks for human health at low dose/low dose rates is an important issue in radiation protection.
Whereas high doses effects are well-characterized, those caused by low doses are extrapolated from effects at high dose.
Possible extrapolations of radiation-induced cancer risk to doses where epidemiology cannot go

- Supra-linearity
- Linear
- Linear quadratic
- Infra-linearity (hormesis)
A BEIR VII Conclusion

Knowledge on adaptive/hormetic responses and their underlying mechanisms, which may act to alter radiation cancer risk was judged to be insufficient to be incorporated into modeling of epidemiological data.
The Adaptive Response is a phenomenon induced by low dose/low LET radiation that protect cells and whole organisms against endogenous damage or damage due to a subsequent dose of radiation.
“Adaptive response of human lymphocytes to low concentrations of radioactive thymidine”
Olivieri G, Bodycote J, Wolff S.

<table>
<thead>
<tr>
<th>Treatment</th>
<th># Genetic Lesions</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>$[^3]$H$d$Thd (0.1 $\mu$Ci/ml)</td>
<td>5</td>
</tr>
<tr>
<td>150 cGy</td>
<td>36</td>
</tr>
<tr>
<td>$[^3]$H$d$Thd (0.1 $\mu$Ci/ml) + 150 cGy</td>
<td>13</td>
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</tbody>
</table>
Normal Human Fibroblasts Growing in a Three-Dimensional Architectural Environment (to mimic \textit{in vivo} growth)
Effect of a priming dose of $\gamma$-rays (0.2 cGy/h) on modulation of the cellular response to a subsequent acute challenge dose.

Protective responses are observed following exposure to adapting doses as low as 1 cGy.
\( \gamma \)-ray induced adaptive responses are transient
Mechanisms Underlying Low Dose $\gamma$-Ray Induced Adaptive Responses
Oxidative metabolism and DNA repair capacity mediate expression of adaptive responses induced by low dose/low dose-rate γ-rays.
Involvement of **Cell Cycle Checkpoints** in Expression of the Adaptive Response

![Graph showing cumulative labeling index (% labeled) over time after subculture (h). The graph compares different treatments:
- Control
- 1 Gy (3h)
- 10 cGy + 1 Gy (3h)●

The graph demonstrates the effect of different radiation treatments on the cumulative labeling index over time.
Other Mediating Mechanisms

• Oxidative metabolism
• Up-regulation of DNA repair
• Induction of cell cycle checkpoints
• Intercellular communication
• Apoptosis
• Altered chromatin conformation
Effect of a Single low Dose of $\gamma$-Rays Delivered at Various Dose-Rates on DNA Damage in Normal Human Cells Growing in 3-D

The Role of Oxidative Metabolism: Exposure to Low Dose/Low Dose Rate $\gamma$–Rays Up-Regulates the Antioxidant Glutathione

Fold increase in intracellular glutathione concentration

- Control
- 10 cGy acute
- 10 cGy / 2 h
- 10 cGy / 24 h
- 10 cGy / 48 h

1.2 - 1.6 fold
Adaptive Responses and oxidative metabolism

- Up-regulations of superoxide dismutase
- Up-regulation of thioredoxin
- Modulation of NF\textsubscript{KB}
Low Dose $\gamma$-Rays and Gene Expression

Our cDNA microarray & proteomics data indicate upregulation by low dose of genes related to aa activation, translation machinery and down-regulation of genes involved in glycolytic pathway.

They reveal up-regulation in mitochondria of redox sensitive pro-survival proteins by low dose/low dose rate and *not* high dose/high dose rate $\gamma$-rays.
Low Dose $\gamma$-Rays & Modulation of mitochondrial function

- 13 proteins
- 22 tRNAs
- 2 rRNAs
- mtDNA
- Outer Membrane
- IMS
- Inner Membrane
- Matrix
- (Signal processing)
- TIM
- TOM
- NH2
- COOH

~900 proteins
High dose $\gamma$-rays decreases mitochondrial protein import and mitochondrial membrane potential

Pandey et al., 2006
Low dose $\gamma$-rays increases mitochondrial protein import and mitochondrial membrane potential

Pandey et al., 2006
Oxidation/reduction reactions induced by low dose rate ionizing radiation are similar to those caused by endogenous metabolism.
Low Dose $\gamma$-Rays and Risk

Effects in Progeny cells

*Induced stress (Physiological or genetic change) is of significance to risk assessment only if it persists and is transmitted to daughter cells.*
Cloning Efficiency of the Progeny of Irradiated Cells

Passage 3 after exposure

- C
- 0.1 Gy chronic
- 4 Gy acute

Passage 6 after exposure

- C
- 0.1 Gy chronic
- 4 Gy acute
Residual Micronuclei in Progeny of $\gamma$-Irradiated Cells

% Binucleated Cells with Micronuclei

- C
- 10 cGy Acute
- 10 cGy Chronic
- 4 Gy
Adaptive response and Risk of Neoplastic Transformation

Neoplastic transformation assay using mouse embryo cells

Control    Irradiated
Low Dose $\gamma$-Rays Reduces the Spontaneous Transformation Frequency in Mouse Embryo Cells

Azzam et al., 1996
In vitro adaptive responses observed for many biological endpoints:

Chromosomal aberrations
Sister chromosome exchanges
Mutations
Gene expression
Survival
...
Often detected by multiple endpoints in the same experiment

However, above observations are not universal
Propagation of ionizing radiation-induced adaptive responses from exposed to non-exposed cells
Co-Culture of tritiated-thymidine labeled cells and bystander cells

\[ ^3 \text{H-Thymidine Labeled cells} \quad \text{Bystander cells} \]

Co-culture

5:95 Labeled to unlabeled
<table>
<thead>
<tr>
<th>³H-Thymidine Concentration (µCi/ml)</th>
<th>Micronucleus Frequency</th>
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<tr>
<td>0</td>
<td>0.012 ± 0.003</td>
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<tr>
<td>4</td>
<td>0.028 ± 0.005</td>
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<tr>
<td>0.04</td>
<td>0.004 ± 0.001</td>
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These data indicate that protective and damaging effects can be propagated in a bystander manner.

Absorbed dose seems to be a determining factor.
Low Dose $\gamma$-Ray induced Adaptive Responses Correlate with Induction of Connexin 43 (a constitutive protein of gap junctions)

Up-regulated connexin43 localizes in specific membrane Regions
Possible extrapolations of radiation-induced cancer risk to doses where epidemiology cannot go

Cancer Risk

Radiation Dose

Supra-linearity

Linear

Linear quadratic

Infra-linearity (hormesis)
Biophysical argument in BEIR VII

Increases in dose simply increase the probability that a given cell in tissue will be intersected by an electron track which will have a non-zero probability of inducing a biological effect. Therefore, at these very low doses, linearity of response is almost certain
Radiation-induced bystander effect in cell cultures exposed to low fluences of $\alpha$-particles

Genetic changes
Gene expression
Biochemical changes
Survival
Dosimetry

Approximate dose to the cell nucleus from a single track

Gamma

~ 0.1 cGy

Alpha

~ 10-70 cGy
Macroscopic dose from 1 $\alpha$-particle track is $\sim 0.15$ Gy/human fibroblast. The specific energy deposited in a directly hit area (e.g. nucleosome) is several dozen Grays.

Cucinotta et al., 2000
# Acknowledgement

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<tr>
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