A Taste of What’s New and What’s Coming in Low Dose Radiation Research

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Setting the stage: Non-targeted effects of low dose radiation exposure evaluated using the radiation chimera model

BALB/c mice (susceptible to IR-induced mammary tumors)

10 cGy exposure to the BALB/c host 3 days prior to transplant reduces time to tumor formation from implanted, un-irradiated *Trp53* null mammary explants


Tumor frequency at the time of experiment termination; *p*<0.05 vs. sham IR
Genotype of the host alters likelihood of cancer promotion by low dose irradiation:
Irradiated $Tgf-\beta$ heterozygous BALB/c mice do not have accelerated time-to-tumor appearance following implantation of un-irradiated $Trp53$ null mammary explants

Tumor frequency at the time of experiment termination not significant vs. sham IR
A systems biology approach to assessment of responses to low dose/low dose-rate exposure to ionizing radiation

LBNL SFA

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Does genetic variation control biological responses to LDIR and mammary cancer risk?

Backcross mouse strains resistant/sensitive to radiation-induced mammary cancer

Link all phenotypes to genetic loci through SNP analysis
Genotype of the host alters likelihood of cancer promotion by low dose IR: 10 cGy irradiated F1Bx hosts have increased latency and reduced frequency of tumor formation after implantation of non-irradiated *Trp53* null mammary explants.
SNP analyses reveal 15 genetic loci that interact with low dose irradiation (10 cGy) to control tumor latency in radiation chimeras: 4 BALB/c loci are protective, 11 SPRET/EiJ loci are protective*

Table 1: Genetic loci intact with LDIR controlling susceptibility to mammary tumor development

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<tr>
<th>Chromosome</th>
<th>Location (Mb)</th>
<th>SNP ID</th>
<th>Homozygous</th>
<th>Heterozygous</th>
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</table>

*HR= Hazard Ratio for 10cGy vs Sham.  #CI = Confidence Interval

* Reduced hazard ratio indicates protective effect of a given genotype
Candidate plasma cytokines/chemokines associated with delayed/reduced likelihood of development of mammary tumor after 10 cGy exposure to the host and implantation of Trp53 null mammary explants.
Interactions of the host environment with low dose radiation: Does the hormonal profile impact low dose radiation responses and mammary tumor risk?

**Approach:** Measure and analyze MG transcriptome and immune system changes during the estrus cycle in mice sensitive to mammary tumorigenesis post-IR (BALB/c) or mice resistant to mammary tumorigenesis post-IR (C57BL/6). Studies performed with and without LDIR (JGI: RNA seq)
Mammary gland gene transcript levels vary with genotype and estrous stage: upregulation of IRF-7 regulated genes in both glands in proestrus, but little or no effect of 10 cGy (not shown).


White = BALB/c; Black = C57BL/6; Gray = common

Example: IFIT1, an IRG-7 regulated gene, goes up in proestrus in BALB/c
Blood lymphocyte studies and plasma cytokine studies: Hormonal regulation and low dose sensitivity in mice sensitive to IR-induced mammary tumors


Sham irradiation:
Only BALB/c PBL counts fluctuate with estrus stage

10cGy reduces PBL counts in BALB/c mice only.

Irradiation in a single phase of estrous cycle

Plasma cytokine changes in BALB/c mice after 10 cGy (not shown).

Irradiation in a single phase of estrous cycle
Novel imaging technologies reveal apparent non-linear yields of radiation-induced foci associated with early DNA damage: MCF10A cells with 53bp1-GFP evaluated by live cell imaging


![Graphs showing time post-IR (hours) vs. Average RIF/Gy/cell for 10 cGy and 100 cGy.](image)
Are risks associated with radiation truly linear with respect to dose?

**Approach:**

- Track cells and repair foci live, through cell division, using microfluidic chamber and ‘miniX’ in-scope irradiator (new device and custom software, S. Costes)
- Use registration algorithms and maximum projection to identify 3D repair sub-domains (RIFs) and changes over time/division
- Use data in Monte Carlo simulations to model how DSB clustering impacts cell death, chromosome rearrangements and dose response

COSMIC SILENCE (SC)

Influence of radiation environment on the metabolism and on the response of living organisms to genotoxic agents

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XLII meeting of the Gran Sasso Scientific Committee – November 13-14, 2014
**COSMIC SILENCE Hypothesis**

Life has evolved on Earth for 3 billion years in the presence of environmental ionizing radiations, able to induce modification/damage at the level of DNA. All organisms are subjected to a daily stimulus consisting of ultra-low-dose environmental radiation that could act as a conditioning agent, making them more efficient in activating DNA repair mechanisms.

**COSMIC SILENCE Aim of the Project**

To investigate the role of environmental radiation on living organisms

i) Evaluation of the impact of environment

ii) Analysis of the modulation of the response to genotoxic agents in biological systems grown in normal or reduced presence of environmental radiation
PARALLEL EXPERIMENTS UNDER DIFFERENT ENVIRONMENTAL RADIATION CONDITIONS

Reference Radiation Environment (RRE) lab

Istituto Superiore di Sanità Rome (external lab)

Low Radiation Environment (LRE) lab

Cosmic rays: $10^6$ reduction factor
Neutrons: $10^3$ reduction factor
CONCLUSIONS

Cells at LNGS LRE lab:
- more sensitive to DNA damage,
- more sensitive to acute-dose radiation,
- reduced ability in ROS scavenging.
Drosophila as animal model

- Short life cycle (development and reproduction)
- High fecundity and high number of offspring
- Suitable for mutagenesis assays
- Small and easy to grow in laboratory
- Low overall costs
Summary 1

- Biological studies have highlighted the role of genetic background in the determination of risks of cancer induction following low dose exposure to ionizing radiation.

- Modern “omics” approaches have identified candidate loci that regulate low dose tumorigenesis in mouse models. These findings may be useful in future molecular epidemiologic studies.

- Coordinated research has also identified candidate biomarkers of exposure at short times after low dose exposure that reflect the influence of genetic background and that are associated with the risk of cancer development at later times.
Advanced imaging technology, notably live cell imaging, has raised questions regarding the interpretation of data generated with static imaging methods to assess low dose response.

Model development provides testable hypotheses regarding cellular responses to ionizing radiation as a function of dose.

This approach may ultimately help refine the shape of the dose-response curve at low doses for key steps along the path to cancer development.

The role of natural background radiation in the regulation of response to subsequent environmental challenges is being examined in a series of well-controlled investigations.