Mechanism underlying individual radiosensitivity of breast and head and neck cancer patients

Siamak Haghdooost
Centre for Radiation Protection Research
Stockholm University
WP6 DoReMi
Individual variability in susceptibility to cancer (WP 6)
The overall objectives for WP6 are

• To provide the experimental evidence to incorporate the influence of genetic modifiers, age and gender on cancer risk, at low doses and low dose rates, to radiation protection practices

• To coordinate activities with cross-workpackage programs in molecular epidemiology, dosimetry and systems biology.

• To integrate WP6 research with activities from national and EURATOM sponsored research into the HLEG roadmap through the preparation of open calls for scientific projects
Description of WP6 tasks

Task 6.1 Molecular epidemiological studies to address the role of individual genetic variation in determining susceptibility to low doses (Starting Year 1).

Task 6.2 Identification of genetic modifiers of individual cancer susceptibility and their mechanisms of action

Task 6.3 Modelling of the effects on risk prediction models due to changes in biological processes influenced by genetic variability

Task 6.4 The effect of genetic modifiers on carcinogenesis following low dose rate exposure

Task 6.5 Contribution of genetic and epigenetic mechanisms that indirectly influence susceptibility to radiation-induced cancer

Task 6.11: RADSENS
Task 6.8

Poster **2-PS2D-12** (Tuesday, May 26\textsuperscript{th})
Investigation of radiosensitivity in healthy controls, AT patients and prostate cancer patients. Presenter: Lisa White

Poster **4-PS1B-52** (Thursday May 28\textsuperscript{th})
Low dose effects in peripheral blood lymphocytes with Raman spectroscopy. Presenter: Dr Jane Bryant
Aims of Radsens:

• What pathways triggered by low dose radiation (mGy range)
• What pathways differing sensitive/non sensitive patients

**Longterm aim:**

• To have predictive assay to be able to distinguish between extreme sensitive normal normal sensitive patients

Cohorts?
**Adverse effects of radiotherapy**

**Acute effects**
- **Fibrosis**: Proliferation of surviving fibrocytes owing to growth factors released as a result of injury. Examples: Dermatitis, Mucositis, Cystitis, Proctitis, Hair loss, Bone marrow suppression.

**Atrophy**: Loss of fibrocytes and collagen reabsorption. Examples: Hardening and shrinkage of an irradiated breast, Strictures and malabsorption of irradiated small bowel.

**Late effects**
- **Vascular damage**: Either small vessel dilation or constriction. Examples: Telangiectasia in the skin, Bleeding, e.g. haematuria, Ischaemia resulting in bowel perforation and formation of fistulae.
- **Infertility**
- **Hormone deficiencies**
- **Second malignancies**

Ex. Osteoradionecrosis

Ex. Severe skin reaction

Validation of candidate biomarkers in an extended cohort of patients
> 200 patients
Breast cancer cohort

Total 2914 patients

S Skiöld et al. 2013 Mut. Res. 30; 756 (1-2): 152-7
The incidence for ORN is ~5%

It is a late adverse effect to radiotherapy occurring 1-10 years after the end of the treatment.

There are ~280 new cases/year of head and neck cancer in Stockholm, Sweden

Available:

37 patients with osteoradionecrosis (ORN) and 37 matched controls.
Radiosensitive group:
High background levels and low therapy related increase of urinary 8-oxo-dG

Non-sensitive group:
Low background levels and high therapy related increase of urinary 8-oxo-dG

P < 0.05

P < 0.001

Intl. J. of Radiation Oncology, Biology, Physics, 2001

Radiosensitive
Collaboration with Dr. Soile Tapio, Germany

**RTOG 0**
- Pooled from 9 donors after IR and protein isolation
- 0 mGy
- 1 mGy
- 150 mGy

- Triplex ICPL labelling comparing the control to the irradiated samples.

**RTOG 4**
- Pooled from 8 donors after IR and protein isolation
- 0 mGy
- 1 mGy
- 150 mGy

- Triplex ICPL labelling comparing the control to the irradiated samples

- Duplex labelling comparing the non irradiated controls of the two groups
Normo-sensitive patients

Radiosensitive patients

8-oxo-dG level

SOD1
CA1
PARK7
PRDX2
SH3BGRL3

BLVRB
PRDX2

Pooled samples
1 mGy gamma radiation affects several protein expression in cells from normosensitive related to:

- Oxidative stress / NRF2-mediated oxidative stress response,

- Decreased transmembrane potential of mitochondria and mitochondrial membrane

Unique proteomic signature for radiation sensitive patients; a comparative study between normo-sensitive and radiation sensitive breast cancer patients

Sara Skiöld\textsuperscript{a}, Omid Azimzadeh\textsuperscript{b}, Juliane Merl-Pham\textsuperscript{c}, Ingemar Naslund\textsuperscript{d}, Peter Wersall\textsuperscript{d}, Elisabet Lidbrink\textsuperscript{d}, Soile Tapio\textsuperscript{b}, Mats Harms-Ringdahl\textsuperscript{a}, Siamak Haghdoost\textsuperscript{a}. Mutation Research. 2014,
DNA repair
OGG1 (1) XRCC3 (2)
ATM (6) RAD21 (1)
RAD9A (2) RAD17 (1)
MTH1/NUDT1 (1) TP53 (1)
XRCC1 (2)

Oxidative stress
NOS3/eNOS (1)
GSTP1 (1)
Catalase (1)
NFE2L2 (1)
SOD2a

Inflammation
TGFB (2)
HIF1A (5)
IL12RB2 (1)
VEGFA (7)

Individual radosensitivity/ORN

Investigating 58 point mutations (SNPs) previously implicated in side effects to RT
Danielsson, D. et al. 2014, head and neck
miRNAs as biomarker for individual radiosensitivity (RADSENS)

miRnome analysis: 1152 miRNAs

<table>
<thead>
<tr>
<th>sample</th>
<th>down, up</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTOG 3/ RTGO 0</td>
<td>20, 11</td>
</tr>
<tr>
<td>RTOG 3, 150 mGy</td>
<td>23, 24</td>
</tr>
<tr>
<td>RTOG 0, 150 mGy</td>
<td>18, 15</td>
</tr>
<tr>
<td>RTOG 3, 1 mGy</td>
<td>61</td>
</tr>
<tr>
<td>RTOG 0, 1 mGy</td>
<td>41, 24</td>
</tr>
</tbody>
</table>
miRNA expression changes after low dose irradiation

**miR-183-5p**

- Box plot showing ΔCT values for different irradiation conditions: RT0G0 con, RT0G0 1 mGy/h, RT0G3 con, RT0G3 1 mGy/h, RT0G3 150 mGy/h.
- Statistical significance: $p=0.09$.

**miR-224-5p**

- Box plot showing ΔCT values for different irradiation conditions: RT0G0 con, RT0G0 1 mGy/h, RT0G3 con, RT0G3 1 mGy/h, RT0G3 150 mGy/h.
- Statistical significance: **$p<0.01$**.
General conclusion

- Patients individual ability to handle oxidative is related to individual response to radiotherapy

- miRNA biomarkers: validation is ongoing in cellular model system using miRNA inhibitors

- Oxidative stress response, coagulation properties and acute phase response are hallmarks of radiation sensitivity supporting our previous study on oxidative stress response.
  - 8-oxo-dG levels
  - proteomic approach
  - SNP in GSTP1
Helmholtz Zentrum München
Deutsches Forschungszentrum für Gesundheit und Umwelt
Dr. Soile Tapio
Dr. Simone Mörtl

CRPR, Stockholm University