Human Radiosensitivity and Prospects for Prediction

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Members of the C1 WG on individual radiosensitivity:

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Michael Hauptman
Simon Bouffler (MC)
Major reports on human radiosensitivity and cancer susceptibility
Many definitions for the term „radiosensitivity“

- Whole organism radiosensitivity
  refers to radiation-related mortality due to deterministic effects

- Normal tissue radiosensitivity or clinical radiosensitivity
  refers to adverse reactions in non-target tissues as consequence of radiotherapy (deterministic effects)

- Normal tissue radiosensitivity to non-cancer, non deterministic effects
  refers to such effects as cataracts and cardiovascular disease

- Susceptibility to radiation carcinogenesis
  refers to susceptibility amongst individuals to radiation-induced cancer

- Tissue radiosensitivity for cancer
  refers to insensitivity of individual tissues to radiation-induced cancer

- Cellular radiosensitivity
  refers to endpoints measured at the cellular level such a DNA damage
The importance of defining the endpoint when talking about individual radiosensitivity

**Children are radiosensitive with respect to stochastic effects**


*ERR for cancer as a function of age and sex in the LSS cohort*

- Radiosensitivity decreases with age

**Children are radioresistant with respect to deterministic effects**


*Median lethal dose as a function of age in mice*

- Radiosensitivity increases with age

**Biological explanation**
- **age effect**: long life expectancy, many cell divisions
- **sex effect**: mainly breast cancer

**Biological explanation**
- Decreasing regenerative capacity of tissues with age
It is often assumed that the individual radiosensitivity and radiosusceptibility is genetically determined and is an „intrinsic“ trait. This is based on the established high radiosensitivity and/or radiosusceptibility of rare diseases associated with impaired DNA repair capacity.


<table>
<thead>
<tr>
<th>Syndromes</th>
<th>Mutated Genes</th>
<th>Major defective mechanism</th>
<th>Cancer predisposition</th>
<th>Clinical sensitivity to Br³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td>ATM, homzygous mutations</td>
<td>DSB signaling and repair</td>
<td>Leukemia, Lymphoma</td>
<td>+++</td>
</tr>
<tr>
<td>Ligase IV</td>
<td>Lig IV, homozygous mutations</td>
<td>NHEJ</td>
<td>Leukemia, Lymphoma</td>
<td>+++</td>
</tr>
<tr>
<td>Nijmegen</td>
<td>NBS1, homozygous mutations</td>
<td>DSB signaling and repair</td>
<td>Leukemia, Lymphoma</td>
<td>+++</td>
</tr>
<tr>
<td>Hutchinson-Gilford (progeria infantum)</td>
<td>Lamin A, homozygous mutations</td>
<td>Nuclear membrane</td>
<td>No</td>
<td>+++</td>
</tr>
<tr>
<td>Bruton’s disease (agammaglobulinemia)</td>
<td>BTK, homozygous mutations</td>
<td>V/D/J recombination</td>
<td>No</td>
<td>+++</td>
</tr>
<tr>
<td>Hypo-gammaglobulinemia</td>
<td>Lig I, homozygous mutations</td>
<td>NER</td>
<td>No</td>
<td>+++</td>
</tr>
<tr>
<td>Glutathione synthetase deficiency</td>
<td>GSS</td>
<td>Glutathione cycle</td>
<td>No</td>
<td>+++</td>
</tr>
<tr>
<td>ICF syndrome</td>
<td>DNMT3B</td>
<td>DNA methylation DSB signaling and repair</td>
<td>No</td>
<td>+++</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>IT15</td>
<td>DNA methylation DSB signaling and repair</td>
<td>No</td>
<td>++</td>
</tr>
<tr>
<td>Neurofibromatosis type I (Von Recklinghausen)</td>
<td>NF1</td>
<td>DSB signaling and repair</td>
<td>Central and peripheral nervous system tumors</td>
<td>++</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>TSC genes</td>
<td>DSB signaling and repair</td>
<td>Central and peripheral nervous system tumors</td>
<td>++</td>
</tr>
</tbody>
</table>

All together, ca 15 disorders are known showing increased cellular radiosensitivity. They are generally the result of low frequency, high penetrance mutations that are not often seen in the general population.
If the individual radiosensitivity and radiosusceptibility is an "intrinsic" trait then the radiosensitivity of cells isolated from an individual should correlate with his/her radiosensitivity and radiosusceptibility

**Functional assays**

- Fibroblasts
- Peripheral blood lymphocytes

**Source:** N. Foray et al. Individual response to ionizing radiation. Mutat Res. 770: 369-386, 2016
Biomarkers of individual radiosensitivity

The horror scenario for a radiation oncologist: skin necrosis – severe late side effect to radiotherapy

1999: RT for Hodgkin's disease
picture taken in 2005

The total dose was 32 Gy in 20 fractions of 1.6 Gy given 5 days a week with 9 MeV photons.

Patient did not show an in vitro radiosensitive phenotype (chromosomal aberrations)
### Small-scale studies using functional assay yield controversial results

**Examples: Residual DNA damage (repair foci) and clinical radiosensitivity**

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>O Nuta et al. <em>Correlation between the radiation responses of fibroblasts cultured from individual patients and the risk of late reaction after breast radiotherapy.</em> Cancer Lett. 374:324-330, 2016.</td>
<td>Residual 53BP1 foci counts 24 h after in vitro irradiation were significantly higher in fibroblasts from RT-sensitive versus RT-resistant patients.</td>
</tr>
<tr>
<td>P. Lobachevsky et al. <em>Compromised DNA repair as a basis for identification of cancer radiotherapy patients with extreme radiosensitivity.</em> Cancer Lett. 383:212-219, 2016.</td>
<td>The most powerful predictor of extreme toxicity was a combination of the fraction of the unrepairable component of γ-H2AX foci and repair rate in PBL.</td>
</tr>
<tr>
<td>M. Chua et al. <em>DNA double-strand break repair and induction of apoptosis in ex vivo irradiated blood lymphocytes in relation to late normal tissue reactions following breast radiotherapy.</em> Radiat Environ Biophys. 53:355-364, 2014.</td>
<td>No association was observed between apoptosis and residual focus levels in breast cancer patient groups with various late toxicities.</td>
</tr>
</tbody>
</table>
Today, GWAS appears to be the best way forward

- Complex diseases or traits are often associated with a specific pattern of SNP variants. Available GWAS results suggest that the same may be true for radiosensitivity. A SNP fingerprint will be specific for each type of late toxicity.
- Currently, several large studies are in progress whose main goal is to discover new SNPs and validate previously identified genetic biomarkers of radiosensitivity.
BUT: there are major confounding factors in identifying markers of radiosensitivity

**Treatment planning** – Significant differences between hospitals/RT professionals in contouring of PTV and organs at risk.

**Dosimetry** — detailed treatment and dosimetric data is essential (DVH) but often lacking. Moreover, some TPS poorly estimate doses to tissues distal to PTV.

*Remember: we may be looking at side effects to a RT carried out many years ago*

**Measures and scales** used to assess adverse effects – different measures and scales are used across hospitals.

**Outcomes** – multiple measures of toxicity for the same outcome are used.

Contours of the internal mammary nodes, the lumpectomy cavity, boost PTV, and the breast volume in an axial plane.


Biomarkers of individual radiosusceptibility

- A factor which contributes to intrinsic cancer susceptibility is the genetic background which is associated with genomic instability leading to an increased level of mutations and to sensitivity to environmental factors.

- Genomic instability can be identified as increased spontaneous or radiation-induced frequency of chromosomal aberrations. The latter is called the Mutagen Sensitivity Assay. Radiation can be substituted by bleomycin (BLM).
However, the fraction of cancers attributed to genetic background is low

Proportion of cancer susceptibility accounted for by genetic factors

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid cancer</td>
<td>53%</td>
</tr>
<tr>
<td>Endocrine glands</td>
<td>28%</td>
</tr>
<tr>
<td>Testis</td>
<td>25%</td>
</tr>
<tr>
<td>Breast</td>
<td>25%</td>
</tr>
<tr>
<td>Cervix</td>
<td>22%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>21%</td>
</tr>
<tr>
<td>Colon</td>
<td>13%</td>
</tr>
<tr>
<td>Nervous system</td>
<td>12%</td>
</tr>
<tr>
<td>Rectum</td>
<td>12%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10%</td>
</tr>
<tr>
<td>Lung</td>
<td>8%</td>
</tr>
<tr>
<td>Kidney</td>
<td>8%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>7%</td>
</tr>
<tr>
<td>Stomach</td>
<td>1%</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>1%</td>
</tr>
</tbody>
</table>


Replication errors in stem cells may be responsible for ca 70% of the mutations in human cancers

Nevertheless: a high chromosomal radiosensitivity of skin fibroblasts is a hallmark of cancer susceptibility

Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 5400–5403, August 1985
Cell Biology

Chromosomal radiosensitivity during the $G_2$ cell-cycle period of skin fibroblasts from individuals with familial cancer

(chromatid gaps and breaks/cell-cycle-related radiosensitivity)

RAM PARSHAD*, KATHERINE K. SANFORD†, AND GARY M. JONES‡

Fig. 1. Comparison of chromatid damage induced by x-irradiation (100 R) during $G_2$ phase of skin fibroblasts from normal donors (a), skin fibroblasts from individuals with genetic disorders associated with a high risk of cancer (b) (13), skin fibroblasts from cancer patients (c), and human tumor cells (d) (9). The genetic disorders represented, in order of increasing chromatid damage, were xeroderma pigmentosum variant, Gardner syndrome (GS), xeroderma pigmentosum, complementation group E (XP-E), GS, Bloom syndrome, XP-C, familial polyposis, ataxia telangiectasia heterozygotes (five individuals) and homozygotes (two individuals) (10, 13). Data on XP-A cells have not been included; for explanation, see ref. 13. The tumor cells were from malignancies of diverse tissues of origin and histopathology (9).
High spontaneous aberration frequency in lymphocytes is a hallmark of cancer susceptibility

Kaplan–Meier curves for total cancer incidence tertile of CA frequency based on pooled data from 11 European cohorts. Cancer-free probability refers to time from CA test to the first cancer diagnosis.

The idea of the study
Find a database with spontaneous aberration scores in lymphocytes of a cohort
Follow up the cohort for cancer incidence/mortality
Correlate the aberration score with RR (calculated as SIR, SMR or HR)

High CA frequency was associated with the risk of stomach cancer.

The presence of chromosome instability stomach cancers may be linked to the metabolisms of agents involved in stomach carcinogenesis, such as folic acid and vitamin B12.

However, *Helicobacter pylori* infection is also known to increase the level of chromosomal damage in lymphocytes.

Lymphocytes of breast cancer patients show an enhanced radiation-induced aberration frequency ($G_2$ test)

Chromosomal radiosensitivity and low penetrance predisposition to cancer


$G_2$ chromosomal radiosensitivity of normal donors and breast cancer patients. The dashed vertical lines indicate the cut-off point between a normal and a sensitive response.
Lymphocytes of patients with some other cancers may also show enhanced radiation-induced aberration frequencies.
Mutagen sensitivity studies suggest a much higher genetic component in cancer susceptibility than epidemiological genetic linkage studies


Table 1. Contribution of genetic and environmental variance components to mutagen sensitivity

<table>
<thead>
<tr>
<th>Variance components</th>
<th>Mutagen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bleomycin</td>
</tr>
<tr>
<td>Genetic contribution (%)</td>
<td>40.7</td>
</tr>
<tr>
<td>Nonshared environment (%)</td>
<td>33.3</td>
</tr>
<tr>
<td>Shared environment (%)</td>
<td>26.0</td>
</tr>
</tbody>
</table>

Abbreviations: BPDE, benzo[a]pyrene diol epoxide; 4NQO, 4-nitroquinoline 1-oxide.
Is a high mutagen sensitivity really a marker of genetically-determined cancer susceptibility?

G. Szekely et al. Does the bleomycin sensitivity assay express cancer phenotype?
Mutagenesis 18: 59–63, 2003

Comparison of PBL sensitivity to BLM in H&N cancer patients, healthy normal people and healthy alcoholics.

No difference between cancer patients and alcoholics.

BLM assay seems to be a tool for characterization of genotoxic exposure to heavy tobacco and alcohol use rather than for individual susceptibility to cancer.
Is a high mutagen sensitivity really a marker of genetically-determined cancer susceptibility?

M. Khosravifarsani et al. The study of radiosensitivity in left handed compared to right handed healthy women. BMC Medical Physics 12:3, 2012

Figure 1 Mean frequency of MN in right handed and left handed women.
Sequential chromosome aberration analysis following radiotherapy — no evidence for enhanced genomic instability

E. Janet Tawn *, Caroline A. Whitehouse, Fiona A. Martin 1

Chromosome analysis in childhood cancer survivors and their offspring — No evidence for radiotherapy-induced persistent genomic instability

E. Janet Tawn a,*, Caroline A. Whitehouse a, Jeanette F. Winther b, Gillian B. Curwen a, Gwen S. Rees a, Marilyn Stovall c, Jorgen H. Olsen b, Per Guldberg d, Catherine Rechnitzer e, Henrik Schroder f, John D. Boice Jr. g, h
Patients with SMN (second malignant neoplasms) appear to be an attractive cohort for studies of biomarkers of cancer susceptibility, BUT:

Problem 1: the dose-response relationship for SMN is not well known

Where do the SMN occur? Dose at the site of origin.

How precise is the dose estimate at the site of SMN? A problem is the long time span between RT and manifestation of SMN

Patients with SMN (second malignant neoplasms) appear to be an attractive cohort for studies of biomarkers of cancer susceptibility, BUT:

Problem 2: the doses received by organs and tissues at risk are poorly defined

Can you reduce your individual radiosusceptibility?
Remember: all risks are conditional

- Cancer risk models recommended for use by the ICRP depend to a large extent on excess relative as opposed to excess absolute risk.
- This suggests that the risk of radiation-induced cancer is to a great extent determined by the same factors that determine cancer risk in the general population.
- Therefore, measures that reduce population cancer risk incidence and mortality should help reduce the incidence of radiation-associated cancer in populations.

- Can the risk of radiation-induced cancer be reduced after a radiation exposure has taken place?
- If this is the case then people who have been exposed to radiation (e.g. due to Chernobyl or Fukushima Daiichi accidents) can - to some extent - control their risk.
- This can have an enormous implication for their well being and, eventually, for their health.
Leisure-Time Physical Activity reduces the Risk of 26 Types of Cancer in 1.44 Million Adults


1.44 million participants (59 [19-98] years), 57% females and 43% males, 186 932 cancers
High vs low levels of leisure-time physical activity were associated with lower risks of 13 cancers:

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Hazard ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophageal adenocarcinoma</td>
<td>0.58</td>
<td>0.37-0.89</td>
</tr>
<tr>
<td>Liver</td>
<td>0.73</td>
<td>0.55-0.98</td>
</tr>
<tr>
<td>Lung</td>
<td>0.74</td>
<td>0.71-0.77</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.77</td>
<td>0.70-0.85</td>
</tr>
<tr>
<td>Gastric cardia</td>
<td>0.78</td>
<td>0.64-0.95</td>
</tr>
<tr>
<td>Endometrial</td>
<td>0.79</td>
<td>0.68-0.92</td>
</tr>
<tr>
<td>Myeloid leukaemia</td>
<td>0.80</td>
<td>0.70-0.92</td>
</tr>
<tr>
<td>Myeloma</td>
<td>0.83</td>
<td>0.72-0.95</td>
</tr>
<tr>
<td>Colon</td>
<td>0.84</td>
<td>0.77-0.91</td>
</tr>
<tr>
<td>Head and neck</td>
<td>0.85</td>
<td>0.78-0.93</td>
</tr>
<tr>
<td>Rectal</td>
<td>0.87</td>
<td>0.80-0.95</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.87</td>
<td>0.82-0.92</td>
</tr>
<tr>
<td>Breast</td>
<td>0.90</td>
<td>0.87-0.93</td>
</tr>
</tbody>
</table>

Leisure-time physical activity was associated with higher risks of:

- Malignant melanoma 1.27; 1.16-1.40
- Prostate cancer 1.05; 1.03-1.08.

Smoking status modified the association for lung cancer but not other smoking-related cancers.

Possible mechanism: stimulation of immune surveillance
Conclusions

Functionality assays to detect individual radiosensitivity yield very conflicting results so their value is doubtful.

Radiosensitivity is a complex trait so SNP analysis by GWAS appears promising for identifying radiosensitive patients – several large studies are ongoing.

Confounders such as variability in contouring the organs at risk and defining the adverse effects need to be reduced in order to better identify radiosensitive patients.

The value of testing for radiosusceptibility in the context of radiological protection of low-dose occupationally exposed individuals is doubtful because of the low contribution of genetic background.

The effect of lifestyle and other factors on risk following radiation exposure (“effect modifiers”) needs to be better understood so that a “cancer reducing” lifestyle is promoted among exposed people. This will allow them to control the risk leading to increased well being and, eventually, improved health.