Refresher course, topic RC-2 Cellular and molecular effects

Non-targeted biological effects of ionising radiation

Oleg V. Belyakov

STUK - Radiation and Nuclear Safety Authority, Helsinki, Finland.

Faculty of Natural and Environmental Sciences, University of Kuopio, Kuopio, Finland.
Contents

1. Introduction: non targeted effects of ionising radiation
2. Bystander effect and genomic instability: evidence and mechanisms
3. Overview of current bystander effect research
4. Hypothesis, summary and possible implications
5. Future trends in non-targeted research
6. Non-targeted effects and radiation protection
7. The way forward, the NOTE project
8. Beyond the NOTE: the MELODI initiative
9. Change of radiobiological, risk and radiation protection paradigms
10. Conclusions and acknowledgements
1. Introduction: non targeted effects of ionising radiation
Targeted and non-targeted effects of ionising radiation

Targeted effects

Classical paradigm of radiation biology

- DNA damage occurs during or very shortly after irradiation of the nuclei in targeted cells
- The potential for biological consequences can be expressed within one or two cell generations

Non-targeted effects

New evidence

- Bystander effect
- Radiation-induced genomic instability
- Low dose hypersensitivity
- Adaptive response
- Abscopal (out-of-field) effects
- Clastogenic factors
- Delayed reproductive death
- Induction of genes by radiation
Target theory

- The *target theory* of radiation induced effects (Lea, 1946) postulates that cells contain at least one critical site or target that must be hit by radiation in order to kill a cell (or produce an effect).

- Therefore, radiation damage *outside* of the target should not cause cell death (effect).

- It is widely accepted that nuclear DNA is the critical target for radiation induced cell death (and not death related effects).
Non-targeted effects of ionising radiation as a new paradigm of radiation biology

The radiation-induced bystander effect is a phenomenon whereby cellular damage is expressed in unirradiated neighboring cells near to an irradiated cell or cells.
Radiation-induced genomic instability is defined as a persistent elevation in the rate of \textit{de novo} appearance of genetic changes within a clonal population.
Non-targeted *versus* targeted effects

- Non-targeted effects do not contradict to "target theory" but increase size of the target in such extent that concept of "target" became meaningless.
- For example, bystander effect increases target *spatially* to the size of cell group, tissue or even organ.
- Genomic instability increases it *temporarily* by prolongation of damage over many cell generations or even transgenerationally.
Need for a new paradigm of Radiation Biology

• Recent evidence for non-targeted effects suggests a new paradigm for radiation biology that challenges the universality of target theory.
• An essential feature of "non-targeted" effects is that they do not require a direct nuclear exposure by irradiation to be expressed and they are particularly significant at low doses.
• This new radiation biology paradigm should cover both targeted (direct) and non-targeted effects of ionising (and possibly non-ionising) radiation.

Number of papers related to radiation induced non-targeted effects, bystander effect and genomic instability referred by Medline

Total number of papers published 1998-2008 is 439
Rationale for the current interest in non-targeted responses

• There is a growing interest in low dose effects.
• Advances in the technical possibilities for precise low dose irradiation such as development of microbeams, imaging and computerized automation.
• Development of more specific and sensitive methods of cellular and molecular biology.
• Change of classic paradigm of radiation biology and challenging the target principle.
2. Bystander effect and genomic instability: evidence and mechanisms
Evidence for radiation induced non targeted effect

- Increased p53 expression in epithelial cells exposed to $\alpha$-particles (Hickman *et al.*, *Cancer Res*, 1994).
- Medium from $\gamma$-rays irradiated cells reduces the survival of unirradiated cells (Mothersill and Seymour, *Radiat Res*, 2001).
- Bystander effect after microbeam irradiation of a single cell (Belyakov *et al.*, *BJC*, 2001).
Contribution of bystander and direct components to the radiation induced damage

Effect

~0.2 Gy

Dose

Total

Direct effects

Bystander effects
Dose response relationship for direct and bystander mutations

Mathematical models of bystander effects

- **State-vector model (SVM)**
  (Schollnberger, et al., *IJRB*, 2002)
  A biomathematical neoplastic transformation model that includes radioprotective bystander mechanisms. The model successfully simulates experimental data.

- **ByStander Diffusion Modell (BSDM)**
  A quantitative model of the radiation-induced bystander effect based on diffusion-type spreading of bystander signal communication between the hit and non-hit cells.

- **3D lattice model**
  A model for bystander effects, with allowance for spatial position and the effects of cell turnover. It assumes a three-dimensional lattice of points and suitable for tissue modelling.
**BaD model**, contribution of bystander and direct component to the radiation induced oncogenesis

Clonogenic cell death measured in human keratinocytes. The whole bar represents the total death after direct exposure. The red portion of the bar represents bystander death measured after exposure to medium from irradiated cells. The remaining death is represented by the blue portion of the bar, giving a value for death not attributable to bystander effect (Seymour and Mothersill, *Radiat Res*, 2000).
Mechanisms of the bystander effects

- Cell type dependent
- Depends on cell proliferative state
- Energy/REDOX metabolism may be involved
- Bystander effect can be induced by low and high LET irradiation
- Different underlying mechanisms
  - Gap junction (GJIC) mediated
  - Medium borne factors mediated
Hypothetical messenger(s)

At least two types of the bystander messenger might exist

Primary
- emitted by targeted cell
- short lived
- unstable
- travels through gap junctions
- water soluble
- non-protein

Secondary
- produced by activated cells
- long lived
- stable
- media borne
- most likely a protein

Long-lived organic radicals
- Antioxidants (thiols)
- Ca\(^{2+}\) or Ip3
- cAMP

Lipid hydroperoxidases
- Death ligand exfoliation
- Cytokines
- TNF-\(\alpha\), TGF-\(\beta\) or IL-1
Medium borne primary or secondary messengers

- Reactive oxygen species (H$_2$O$_2$/O$^{-2}$) have been proposed as possible signals involved in bystander responses (Narayanan, et al., Cancer Res, 1997; Iyer and Lehnert, Cancer Res, 2000)

Secondary electrons cannot be involved in the bystander effect

- In our research we are using charged particles with energies of 3-4 MeV per nucleon.
- Secondary electrons produced by these particles cannot be involved in the bystander effect because of very short range.
- 7 MeV $^4\text{He}^{2+}$ maximal calculated energy of secondary electrons would be $\approx 3.8 \text{ keV}$, which corresponds to a few hundreds of nanometers range. This is much less than size of cell or cell nucleus. Therefore secondary electrons even would not be able to get out of nucleus after it was targeted with microbeam.
- On other hand, hypothetical bystander messenger is proven to be capable of travel for millimeters.
Bystander effect and genomic instability are closely related

- Bystander effect and genomic instability are non-targeted effects of irradiation and might have common mechanisms (Kadhim et al., Mutat Res, 2004).
- Chromosomal instability could be induced in bystander cells (Lorimore et al., PNAS, 1998).
- There is a recent evidence that the bystander effect persists for many generations (Lorimore et al., Cancer Res, 2005).
- This evidence suggests that the initial cross-section for radiation damage is increased by the bystander effect, and cells that are affected by the bystander mechanism may remain at an increased risk of genetic change for many generations.
3. Overview of current bystander effect research
Studies of bystander effects: a *gradual* movement from *in vitro* cell culture towards *in-vivo* system

<table>
<thead>
<tr>
<th>Gray Cancer Institute</th>
<th>CU</th>
<th>STUK</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro</strong>&lt;br&gt;Normal human fibroblasts&lt;br&gt;Broad field irradiation</td>
<td><strong>In vivo like and ex in vivo</strong>&lt;br&gt;Mouse with implanted piece of human skin&lt;br&gt;Microbeam irradiation</td>
<td><strong>In vivo like and ex in vivo</strong>&lt;br&gt;Mouse with implanted piece of human skin&lt;br&gt;Microbeam irradiation</td>
</tr>
<tr>
<td><strong>In vitro</strong>&lt;br&gt;Primary porcine and human ureter explant systems&lt;br&gt;Microbeam irradiation</td>
<td><strong>Ex in vivo</strong>&lt;br&gt;Primary porcine ureter 3D tissue system&lt;br&gt;<em>In situ</em> microbeam irradiation</td>
<td><strong>In vivo like</strong>&lt;br&gt;Artificial human 3D tissue systems&lt;br&gt;Microbeam irradiation</td>
</tr>
<tr>
<td><strong>In vitro</strong>&lt;br&gt;Primary porcine and human ureter explant systems&lt;br&gt;Microbeam irradiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Completed</strong></td>
<td><strong>Completed</strong></td>
<td><strong>In work</strong></td>
</tr>
</tbody>
</table>

*CU*

*STUK*
Rationale

- Radiation effects at the tissue level under normal conditions prove that individual cells cannot be considered as isolated functional unit within most tissues of a multicellular organism.

- Experimental models, which maintain tissue-like intercellular cell signalling and three-dimensional (3D) structure, are essential for proper understanding of bystander effects.

- The main rationale for our research is that the bystander effect is likely to be natural phenomena which should be studied in an *in vivo* like multicellular system with preserved 3D tissue microarchitecture and microenvironment.

- This necessitates moving from *in vitro* cell culture systems to tissue-based systems.
Microbeams are facilities that allow irradiation of individual cells or cell regions with precise numbers of charged particles with micrometer precision (see for example: Randers-Pehrson et al, Radiat Res, 2001; Folkard et al, Int J Radiat Biol, 1997).
Micronucleated and apoptotic cells

Mironucleated AG01522 fibroblasts (A, B) and urothelial cells (C, D), acridine orange staining.

AG01522 fibroblasts (A and B), porcine urothelium explant outgrowth (C).
Studies of bystander effects in AG01522 normal human fibroblasts

- First direct evidence for a bystander effect.
- Micronucleated and apoptotic cells were scored 3 days after irradiation in AG01522 primary human fibroblasts.
- Irradiation of 1 fibroblast among a few hundred cells with 1\(^{3}\text{He}^{2+}\) particle produced a significant rise in damaged cells from approximately 1% to 3% in the surrounding unirradiated population.
- Further increase of dose does not change the dose response.


Bystander effect in human fibroblasts after $^3$He$^{2+}$ microbeam and ultra soft X-ray microprobe irradiation of a single cell

Porcine ureter section

4 μm paraffin section, Haemotoxylin-Eosin staining
Ureter tissue microarchitecture

Lamina propria

Basal cell layer, dividing

2-3 intermediate cell layers - semi-differentiated, non-dividing

Superficial cell layer - differentiated

Cell movement

Lumen
Primary explant technique

Outgrowth is a 2D representation of 3D tissue microarchitecture including *in vivo* like differentiation pattern.
A proliferation-dependent bystander effect in urothelial explants

• A significant bystander-induced effect was observed only when the periphery of the explant outgrowth (consisting of proliferating cells) was targeted.

• Approximately 2000-6000 additionally damaged cells were produced after irradiation of a few cells initially.

• This finding suggests a cascade mechanism of cell damage induction.

• The fraction of damaged cells did not exceed 1-2% of the total number of the cells within the explant outgrowth.

• The bystander-induced damage depends on the proliferation status of the cells and can be observed with this in vivo like explant model.

Fraction of damaged cells after microbeam irradiation at the periphery of urothelial explant outgrowth, 10 cells have been irradiated at the edge of each explant (10 $^3$He$^2+$ particles/cell)
Bystander-induced differentiation in porcine ureter tissue models following *in situ* microbeam irradiation

- A single 2 μm location on ureter tissue section was pre-irradiated with **10 3He2+ particles** (5 MeV; LET 75 keV/μm).
- Differentiation was estimated using antibodies to **Uroplakin III**, a specific marker of terminal urothelial differentiation.
- Micronucleation and apoptosis involve only a small fraction of cells (typically 1-2% of total cell number).
- Irradiated samples demonstrate about 10-15% additional differentiation in comparison to control. By far the biggest bystander response has a protective role rather than a damaging one by switching on differentiation.

Markers of urothelial differentiation

Porcine explant outgrowth stained with DBA-FITC (A) Uroplakin III staining of porcine ureter section (B) and cells within explant outgrowth (C).
Fraction of differentiated cells measured with Uroplakin III immunostaining in porcine urothelial explant outgrowths

Error bars represent standard error of the means. Significance tests were made using Student’s t-test (*P<0.05; ** P<0.01).
Artificial human skin tissue system

Scheme of human skin

- Epidermis
- Dermis
- Subcutaneous layer
- EpiDerm, EPI-200

EpiDermFT

Scheme of epidermis

- stratum corneum
- hyaline layer
- stratum granulosum
- stratum spinosum
- stratum basale
- basal membrane
Cultivation

Schematic representation of the Air-Liquid Interface tissue culture technique

- Tissue culture well
- Tissue
- Culture insert
- Membrane
- Medium

EpiAirway (AIR-100-SNP)

EpiDerm (EPI-212)
Distance-dependent assay after microbeam irradiation

Paraffin histological section preparation

- Incubation for 1-3 days.
- Fixation in 10% neutral buffered formalin.
- Tissue is cut in half along line of irradiation.
- Paraffin embedding.
- Sample is to be cut in series or levels along X axis.

Microbeam irradiated line or spot in the centre

5 µm paraffin sections
Bystander apoptosis

Bystander induced apoptosis in artificial human skin systems stained with Derma TACS apoptosis kit. Positive apoptotic cells appear blue.

- EPI-201 (A)
- EPI-200-3s (B)
- EPI-200 (C)
- EFT-100 (D)
Bystander effect propagates up to 1 mm away from the irradiated site

- Artificial skin models were irradiated along a straight line across tissue sample (8 mm) every 100 (or 20) μm with α-particles (~7.2 MeV).
- Fractions of micronucleated and apoptotic cells were estimated.
- Mean fraction of bystander apoptotic cells was 3.7±0.6% in irradiated cells and 1.3±0.3% in control.
- Using distance-dependent assay we demonstrated for the first time that bystander effect can be propagated up to 1 mm in tissue after irradiation with α-particle microbeam.

Bystander apoptosis in EPI-200 artificial human tissue after microbeam irradiation
Experimental setup

• Microbeam irradiation of a single 2 µm spot with protons and $^3$He$^{2+}$ ions.
• *In situ* apoptosis assay with 3’-OH DNA end-labelling based technique.
• Studies of bystander-induced differentiation under *in situ* conditions using morphological measurements in underdeveloped EPI-201 model.

EPI-200, 4 µm paraffin section, 3’ OH DNA end-labelling, positive apoptotic cell are green, fluorescent microscope.
Dose-effect dependency for bystander induced apoptosis in EPI-200 artificial human skin models after microbeam irradiation with protons.
Bystander apoptosis in EPI-200 artificial human skin after spot microbeam irradiation with 10 protons
Changes in bystander differentiation pattern after microbeam irradiation EPI-201, 3 days after irradiation

- **Cornified layer** (terminally differentiated cells)
- **Malpighian layer** (non-differentiated, live cells)
Microbeam irradiation increases ratio “cornified layer / total thickness”
MatTek artificial tracheal/bronchial epithelial tissue system

4 μm paraffin section, Haematoxylin - Eosin staining
Bystander induced apoptosis following line $^3\text{He}^{2+}$ microbeam irradiation

Line irradiation, 21 irradiation points along diameter of the tissue. Sections were located approximately 300 $\mu$m away from irradiated spot.
Bystander induced apoptosis following single spot $^3\text{He}^{2+}$ microbeam irradiation

Sections were located approximately 300 $\mu$m away from irradiated spot.
Bystander induced apoptosis following line and spot $^3\text{He}^2+$ microbeam irradiation

![Graph showing fraction of apoptotic cells against number of particles per point (3He)]
4. Hypothesis, summary and possible implications
Hypothesis - bystander effect is a protective mechanism

- Remove potentially damaged *functional group* of cells to decrease risk of transformation.
- Maximal at low doses when a small fraction of cells is exposed.
- Normal tissue *microarchitecture* amplifies the response.
- *Apoptosis* is an important contributor.
- *Irreversible differentiation* is a major pathway of removing potentially damaged cells from proliferating population.
A general scheme of radiation induced bystander effect in tissue systems

Sparse irradiation → Bystander signal → Tissue response

- Track
- Intercellular communication

- Targeted cell
- Potentially damaged cell

- Premature differentiated cell
- Apoptotic cell
Summary

- Bystander response measured as increase in apoptosis, and differentiation was observed in cell cultures, explants and 3D tissue models.
- Bystander induced apoptosis is propagated over large distances in 3D tissue.
- Tissue sample acts as a single unit in response to microbeam irradiation. A cascade mechanism of bystander effect induction might be involved.
- It is tempting to suggest that the bystander response has the function of eliminating potentially damaged cells in the vicinity of radiation induced DNA damage by apoptosis and increased differentiation.
Implications for Radiation Protection

• Non-targeted effects could be important in several radiation related areas.
• It might contribute to better estimation of cancer risk from domestic radon exposure and uranium in drinking water.
• Effects of HZE (high-charge-and-energy) particles during space missions.
• High energy radiotherapy outcome.
• Health effects of air crew and nuclear power station personnel.
• In particular, bystander effect is potentiality significant for radiation protection issues and may have implications for the applicability of the Linear-No-Threshold (LNT) model in extrapolating radiation risk data into the low-dose region.
Significance of the bystander effects for radiotherapy

- The spectrum of secondary malignancies in radiotherapy patients may suggest some contribution of the bystander effect (Hall, Cancer J, 2000).
- Microbeam radiation therapy (Thomlinson, et al., Cell Mol Biol (Noisy-le-grand), 2000) is a new technology of cancer treatment, which might utilise non-targeted effects.
- Finding of a significant bystander induced differentiation after microbeam irradiation would suggest a potential value of the bystander effect for differentiation therapy of cancer treatment; see review of (Beere and Hickman, Anticancer Drug Des, 1993).
5. Future trends in non-targeted research
Experimental systems: opportunities

Currently available

- Primary explant techniques
- Artificial human skin tissue systems
- Tissue scaffolding
- ...

Future directions

- Adaptation of the “window chamber technique” for radiobiological experiments
- Tissue transplants, for example, piece of human tissue grafted on a nude mice
- ...


Tissue scaffolding

- Allows to use conventional cells cultures to form tissue-like 3D microarchitecture.
- Easy to handle, cells could be easily inoculated and extracted with conventional cell culture techniques.
- Preparation of histological sections and non invasive 3D deep tissue imaging is possible.
- Stable, highly reproducible model.

The BD Three Dimensional (3D) Scaffolds: 3D Calcium Phosphate Scaffold (left), 3D Collagen Composite (centre) and OPLA® (Open-Cell Poly-Lactic Acid [right]) scaffolds.
Endpoints

- All models are suitable for histological examination and consequent histoimmunochemistry.
- Deep tissue non-invasive imaging techniques are under development (confocal, 3-photon imaging, Zeiss ApoTome systems).
- Non-destructive life tissue examinations are possible.
- Mutations (?) and epigenetic changes.
- Genomic instability and bystander effect.
- Markers of proliferation and differentiation.
- Malignant conversion (?)
- Progression to invasive cancer (using transformed cell lines and tissue scaffolding or co-culture techniques).
Non-invasive deep tissue imaging

Non-invasive deep fixed and unfixed tissue imaging using Zeiss ApoTome system.
Priorities

- The main priority is a shift from \textit{in vitro} cell systems towards \textit{in vivo} (or at least 3D) tissue models.
- Possible use of human \textit{cell lines} (with tissue scaffolds), tissue transplants, window chambers technique and other \textit{in vivo} human model systems.
- \textit{Low dose irradiation} can be performed with broad and microbeam charged particle and X/\textit{γ}-ray facilities.
Constraints

• Significant *inter-individual variability* (in case of explants).
• Tissue models typically contain *several types of cells*, role of *tissue microenvironment* is significant.
• Cells in tissues are in different *proliferation* and *differentiation* states.
• 3D tissue *difficult to irradiate* quantitatively with existing charge-particle microbeams because of low range (typically tenths of micrometers).
• 3D tissue studies would require new methods of *non-invasive deep tissue imaging* to preserve 3D microarchitecture and study spatial distribution.
6. Non-targeted effects and radiation protection
System of radiation protection

- Present estimations of radiation risk is based on direct epidemiological evidence, as well as on radiation biology research.
- The system is designed to protect against both deterministic and stochastic effects.
- Linear-Non-Threshold (LNT) model is used for estimation of long-term health effects including carcinogenesis and genetic effects.
- A dose and dose-rate correction factor is used to relate the effects of acute exposures to chronic exposures (DDREF).
- Radiation dose is used as a surrogate for risk.
- The effects produced by different types of radiation are assumed to be qualitatively the same.
- Doses can be summed to predict overall risk.
Challenges of the present radiation protection system

• The main objective of the system is to protect the individual. The protection system is generally applicable, in the same fashion, to all age groups, males and females.

• The protection system include the principles of justification, optimisation and exposure restrictions.

• There is a broad international agreement among governmental bodies that the current system of radiation protection is effective, robust and adequately protects people and the environment.

• There are, however, scientific challenges that may bring into question various aspects of the current approach, and which may have significant policy, regulatory and operational implications.

• These challenges include non-targeted effects.
LNT and uncertainties in extrapolation of radiation risk

Risk

Dose

LNT

Epidemiological risk data
Key question

Do non-targeted effects increase or decrease low dose risk in relation to LNT?
The bystander effect might be harmful

• The bystander-induced mutagenesis
  Nagasawa and Little, *Rad Res*, 1999

• Bystander-induced transformation

• Chromosomal instability could be induced in bystander cells
  Lorimore *et al.*, *PNAS*, 1998
  Watson *et al.*, *Cancer Res*, 2000
The risk at low doses might be greater than predicted by LNT.
The bystander effect might be protective

- A gross bystander induced differentiation in the urothelial explant outgrowth after microbeam irradiation
  
  Belyakov et al., Mut Res, 2006

- Cell survival is increased after treatment with medium from irradiated cells
  
  Matsumoto et al., Radiat Res, 2001

- Increase in cell proliferation after low doses of α-particle exposure
  
  Iyer and Lehnert, Cancer Res, 2000

- Bystander effect is a mechanism of tissue integrity maintenance

  Barcellos-Hoff and Brooks, Rad Res, 2001
The risk at low doses might be less than predicted by LNT
## Summary

<table>
<thead>
<tr>
<th>Bystander effects:</th>
<th>RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell death</td>
<td>-</td>
</tr>
<tr>
<td>mutation</td>
<td>-</td>
</tr>
<tr>
<td>chromosomal damage</td>
<td>-</td>
</tr>
<tr>
<td>malignant transformation</td>
<td>-</td>
</tr>
<tr>
<td>premature differentiation</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other non-targeted effects:</th>
<th>RISK</th>
</tr>
</thead>
<tbody>
<tr>
<td>genomic instability</td>
<td>-</td>
</tr>
<tr>
<td>adaptive responses</td>
<td>-</td>
</tr>
</tbody>
</table>
Implications for radiation protection

• The observation of the non-targeted effects are preliminary in nature, and the applicability of any conclusion derived from in vitro studies to in vivo situation is still uncertain.

• The risk at low doses might be greater or less than predicted by a linear extrapolation of the high dose.

• However, non-targeted effects will clearly result in an overall risk, which is a non-linear function of dose.

• It would be premature to consider revising current risk calculations on the basis of current studies of bystander phenomena.

• On other hand, the LNT model is important for radiation protection as a simple method to optimise procedures and regulations. However, it should not be mistaken as a scientific model directly derived from the present state of knowledge of the processes involved in radiation risk estimations.
7. The way forward: the NOTE project
Non-targeted effects of ionising radiation

*European Integrated project, 2006-2010*
NOTE team: 20 partner organisations from the EU, Norway and Canada, **133** scientists and **6** advisers.

**NOTE 1st annual meeting, 17-20 September 2007,**
**Aldemar Knossos Royal Village Hotel, Crete, Greece.**

Non-targeted effects of ionising radiation
Non-targeted effects of ionising radiation

WP1: Management activities
  - Management board
  - Administrative coordinator: Sisko Salomaa
  - WP leaders
  - Advisory committee

WP2: Infrastructures, training and mobility
  - Kevin Prise

WP3: Mechanisms of non-targeted effects
  - Eric Wright

WP4: Non-cancer diseases
  - Guido Hildebrandt

WP5: Factors modifying non-targeted responses
  - Munira Kadhim

WP6: Modelling of non-targeted effects
  - Mark Little

WP7: Dissemination and exploitation activities
  - Oleg Belyakov

- Task 1: Communication with public
  - Riikka Laitinen-Sorvari
- Task 2: Relevance for radiation protection
  - Sisko Salomaa
- Task 3: Conceptualisation of new paradigm
  - Oleg Belyakov
General objectives of the NOTE IP

- To investigate the mechanisms of non-targeted effects, in particular, bystander effects, genomic instability and adaptive response.
- To investigate if and how non-targeted effects modulate the cancer risk in the low dose region, and whether they relate to protective or harmful functions.
- To investigate if ionising radiation can cause non-cancer diseases or beneficial effects at low and intermediate doses.
- To investigate individual susceptibility and other factors modifying non-targeted responses.
- To assess the relevance of non-targeted effects for radiation protection and to set the scientific basis for a modern, more realistic, radiation safety system.
- To contribute to the conceptualisation of a new paradigm in radiation biology that would cover both the classical direct (DNA-targeted) and non-targeted (indirect) effects.
NOTE website: http://www(note-ip.org)
NOTE newsletters

NOTE Newsletter

Issue 2; 14 December, 2007

In this issue

• NOTE DIP2 highlights
• 1st NOTE Annual meeting
• Future meetings
• OECD-NEA workshop
• 2nd Systems Biology workshop
• New paradigm workshop
• 3rd European IRPA Congress in June 2010
• Collaboration
• Periodic Reporting to EC

NOTE DIP2 highlights

Final revised version of the DIP2 - Detailed Implementation Plan for the months 13-30 (1 September, 2007-28 February, 2008) was prepared and submitted to the EC on 7 December, 2007. Addressing low doses and promoting experimentalism - modeller interaction continue to be important themes. The NOTE Management Board will develop a strategy for moving towards the new paradigm and this will be also reflected in the next internal RTD call. The paradigm workshop in Ireland in autumn 2008 will be a major milestone for NOTE. Read more on the highlights of DIP2:

Editor's NOTE

1st Annual review of the NOTE Integrated Project took place 20 November 2007 in Brussels, Belgium. According to Dr. George-Hale Kelly, EC project officer: “The review process went very well and there was a broad consensus that the project is proceeding extremely well”.

In the review process, the Commission was assisted by following independent experts: Prof. William H. Morgan, University of Maryland, USA; Prof. Dudley Goodhead, MRC Medical Research Council, UK and Dr. Wolfgang Weiss; Federal Office for Radiation Protection, Germany.

From the NOTE side Management Board

Next newsletters: months 26, 30, 36 and 42 during DIP3.
NOTE press releases

Workshop on Science and values in radiation protection in Helsinki

Radiation and Nuclear Safety Authority of Finland (STUK) will arrange a workshop on "Science and Values in Radiological Protection" on January 15-17, 2008 in Helsinki in cooperation with the Nuclear Energy Agency (NEA), a specialised agency within the Organisation for Economic Co-operation and Development (OECD).

In the workshop scientists, researchers, authorities, political decision-makers and other experts from 22 countries will gather together to discuss new trends of radiation protection. The scientific knowledge on effects of radiation is increasing continuously and at the same time the values of the society and the demands made on radiation protection are rapidly changing.

The authorities and policy makers responsible for radiation protection must have the best possible knowledge at hand all the time to make valid decisions. On the other hand, the scientists should be able to cooperate with the authorities and the decision-makers in order to provide up to date knowledge on the issue.

The Research Director of STUK, Prof. Sisko Salomaa, is the Chairman of the Organizing Committee of the workshop to be held in January in Helsinki. She states that mutual understanding on the scientific evidence and the radiation protection practice is important both for obtaining optimal protection and for identifying the gaps in knowledge that are most relevant for radiation protection.
44 papers published/accepted in 2006-2008

Research peer reviewed publications


8. Beyond the NOTE: the MELODI initiative
“High Level and Expert Group” (HLEG) on European Low Dose Risk Research

- Formulate and agree the policy goals to be addressed.
- Develop a strategic research agenda and road map.
- Specify elements of and next steps for establishing a sustainable operational framework for low dose risk research in Europe.
- Final report will be published in January 2009 taking account of comments.
- The next step would be establishment of governance structure and detailed Strategic Research Agenda (SRA) and the road map.
Multidisciplinary European LOw Dose Initiative (MELODI)

Low dose Risk Uncertainties

- Analytical Epidemiological Approach
  - Monoclonal Theory of Cancer

Recent Paradigms in Radiobiology
  - (Bystander effects, Genetic instability)

Multicellular Network Biology
  - Systems Biology

Emerging Paradigms in Carcinogenesis

Chronic Exposure
  - (Radionuclides)

Tracer Biology
  - (Radionuclides)

Multiscale Modelling Technics

Track Analysis / Microdosimetry

MELODI STRATEGY

2009

2030

Time
9. Change of radiobiological, risk and radiation protection paradigms
“Scientific paradigm” and “paradigm shift”

Scientific paradigm

- Kuhn introduced the term *paradigm*, which he described as essentially a set of basic statements shared by scientists or a set of agreements about how problems are to be understood.
- Paradigms are **essential** to scientific inquiry.
- A paradigm *guides* the research efforts of scientific communities, and it is this criterion that most clearly identifies a field as a science.
- The typical developmental pattern of a mature science is **the successive transition from one paradigm to another** through a process of revolution.
Development of science is cyclic

Normal science

Scientific crisis

Scientific revolution
Development of science is cyclic
Development of science is cyclic
Paradigmatic changes in radiation biology, radiation risk and radiation protection

- This distinction was introduced recently by Prof. Sisko Salomaa in a document, describing NOTE project research strategy.
- There are different paradigms of radiation biology, radiation risk and radiation protection.
- Radiobiological paradigm describes how radiation acts on cells and tissues, it centers on phenomenology and mechanisms.
- Risk paradigm is connected with qualitative and quantitative estimation of radiation induced health effects, it is based mainly on epidemiological evidence.
- Radiation protection paradigm is a pragmatic system for protection of public and environment from harmful effects exposure to ionising radiation, it is based not only on science but on values as well.
10. Conclusions and acknowledgements
Conclusions

• The current system of radiation protection is robust and protect people well from deterministic and stochastic effects of ionising radiation.
• However, recent discovery of non-targeted effects of ionising radiation indicates that the current radiation protection might be too conservative.
• Linear-Non-Threshold (LNT) model is challenged by non-targeted effects of ionising radiation.
• Health risks associated with non-targeted effects seems to be non-linear.
• Non-targeted effects is constituted paradigm shift in radiation biology, however, respective changes in risk and radiation protection paradigms might take future 20-30 years.
• For that more specific targeted research will be required.
Acknowledgments

National Institutes of Health, USA
US DOE Low Dose program
European Commission
5th and 6th Framework Programmes
Marie Curie Actions
RISC-RAD Integrated project
NOTE Integrated Project
Gray Cancer Institute, UK
Dublin Institute of Technology, Ireland
RESC, Dublin, Ireland
Columbia University, New York city, USA
Center for Radiological Research,
MatTek Corp., Boston, USA
STUK - Radiation and Nuclear Safety Authority, Finland