Studies of bystander effects in human artificial 3D tissue systems following charged particle microbeam irradiation

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Targeted and non-targeted effects of ionising radiation

Targeted effects

Classical paradigm of radiation biology

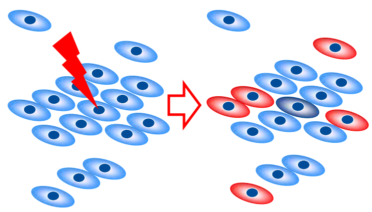
- DNA damage occurs during or very shortly after irradiation of the nuclei in targeted cells
- The potential for biological consequences can be expressed within one or two cell generations

Non-targeted effects

New evidence

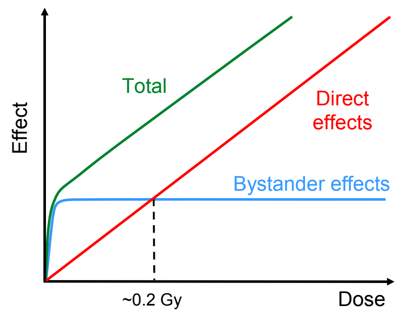
- Bystander effect
- Radiation-induced genomic instability
- Low dose hypersensitivity
- Adaptive response
- Abscopal (out-of-field) effects
- Clastogenic factors
- Delayed reproductive death
- Induction of genes by radiation

Definition



The radiation-induced bystander effect is a phenomenon whereby cellular damage is expressed in unirradiated neighboring cells near to an irradiated cell or cells

Contribution of bystander and direct components to the radiation induced damage



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.

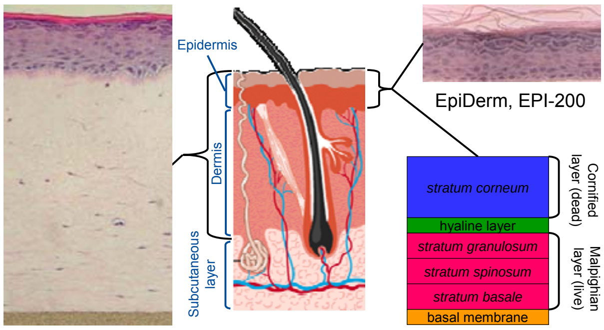
Aims

- Our current work relates to human artificial epidermal (EpiDerm) and trachea-bronchial (AirWay) 3D tissue systems.
- Develop methods of low dose irradiations of 3D tissue models with microbeam irradiation facilities.
- Develop and test protocols for studying endpoints of bystander effects in 3D tissue environment.
- Estimate bystander induced apoptosis and premature differentiation in 3D tissue systems.
- Study mechanisms of bystander effects after microbeam irradiation.
- Clarify the nature of bystander effect and estimate potential applications for radiation protection.

Microbeam technology as a tool for bystander research

Microbeams are facilities that allow irradiation of individual cells or cell regions with precise numbers of charged particles with *micrometer* precision.

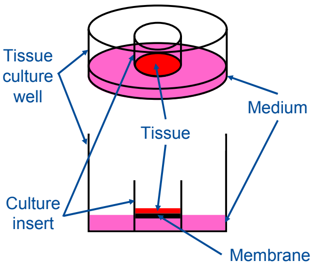
Artificial human skin tissue system



EpiDerm, EPI-200

EpiDermFT — Scheme of human skin — Scheme of epidermis

Cultivation



Schematic representation of the Air-Liquid Interface tissue culture technique

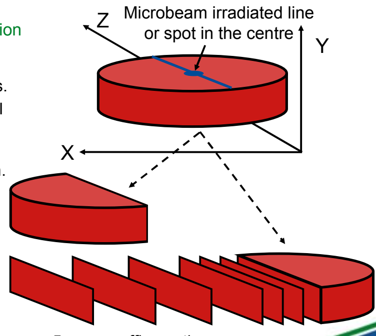
MATTEK CORPORATION

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.
- One half is to be pilot cut in series or levels along X axis.
- Second half is to be stored for future experiments.



5 μm paraffin sections

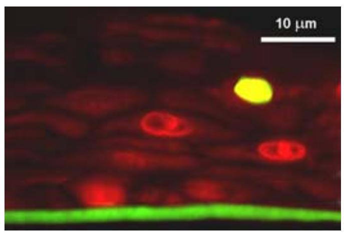
Bystander effect propagates up to 1 mm away from the irradiated site

- Artificial skin models were irradiated along a straight line across tissue sample (8 mm) every 100 (or 20) μм 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.

Belyakov, O.V., Mitchell, S.A., Parikh, D., Randers-Pehrson, G., Marino, S.A., Amundson, S.A., Geard, C.R. and Brenner, D.J. (2005) Biological effects in unirradiated human tissue induced by radiation damage up to 1 mm away. *Proc Natl Acad Sci U S A*, **102**:40, 14203-8.

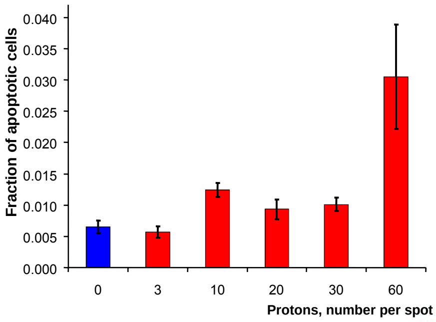
Experimental setup

- 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.

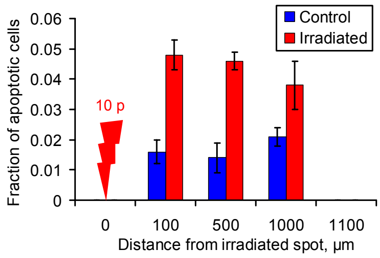


EPI-200, 4 μm paraffin section, 3' OH DNA end-labelling, positive apoptotic cell are green, fluorescent microscope.

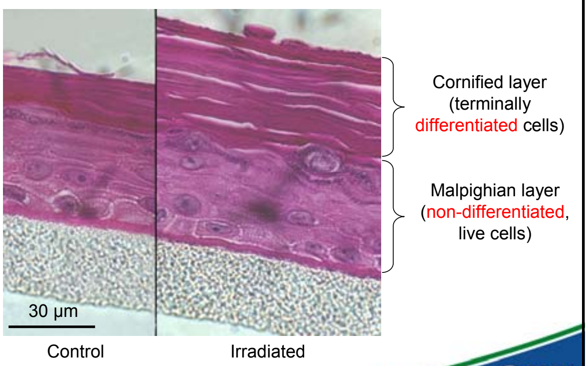
Dose-effect dependency for bystander induced apoptosis in EPI-200 artificial human skin models after microbeam irradiation with protons



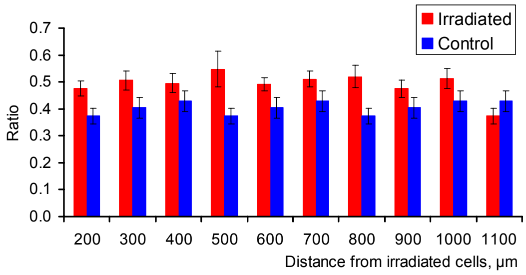
Bystander apoptosis in EPI-200 artificial human skin after spot microbeam irradiation with 10 protons



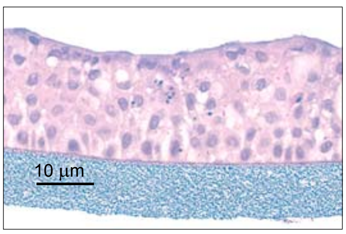
Changes in bystander differentiation pattern after microbeam irradiation EPI-201, 3 days after irradiation



Microbeam irradiation increases ratio “cornified layer / total thickness”



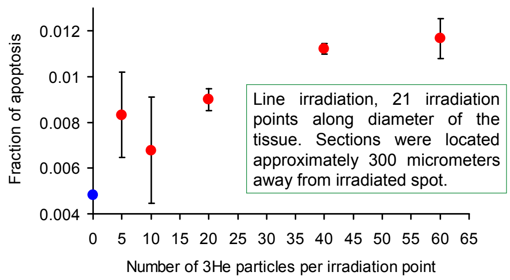
MatTek artificial tracheal/bronchial epithelial tissue system



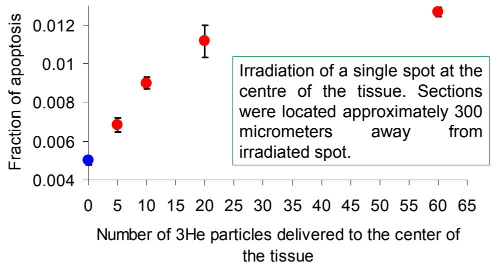
MatTek CORPORATION

4 µm paraffin section, Haematoxylin - Eosin staining

Bystander induced apoptosis following line $^{3}He^{2+}$ microbeam irradiation



Bystander induced apoptosis following single spot $^{3}He^{2+}$ microbeam irradiation



Future work

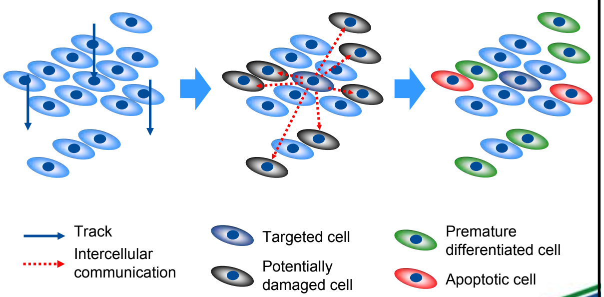
- Mathematical modelling of bystander effect in 3D using an approach developed by Dr. Mark Little (ICFM).
- STUK together with QUB and ICFM are preparing a proposal for collaborative project "TissueMod".
- We will use non-invasive deep tissue imaging technique using Zeiss ApoTome system for non-destructive detection of apoptosis in 3D tissues.
- Time-course of bystander induced apoptosis production will be studied in 3D.
- Mathematical modelling of bystander effect in 3D will help to clarify dynamics of the process and eventually contribute to estimation of risk for radiation protection.

Little M.P., Filipe J.A., Prise K.M., Folkard M., Belyakov O.V. (2005) A model for radiation-induced bystander effects, with allowance for spatial position and the effects of cell turnover. Journal of Theoretical Biology, 232:3, 329-338

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



Conclusions

- 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

- Bystander 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.

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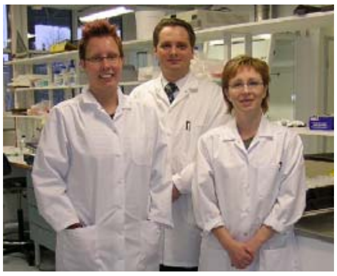

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