



## 7.9 Radiation-induced bystander effects - investigation of secreted factors

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### Background:

Considerable evidence has accumulated recently suggesting that bystander effects can occur in cells which were never themselves irradiated but were either in close proximity to irradiated cells or had received signals from irradiated cells. So far, research by the RESC and others has suggested that radiation causes hit cells to produce signals, which can be received by cells close to or distant from the targeted cell. Responses recorded to date include initiation of apoptosis, differentiation or proliferation.

Despite intense research over the last 5-10 years, the mechanisms underlying radiation induced bystander effects are as yet unclear and the identity of the bystander factor is still unknown. Many groups have attempted proteomics approaches but have been unable to find any new proteins secreted into the irradiated cell conditioned medium.

In addition to soluble proteins, secretion can occur via vesicles termed exosomes. Exosomes are small cup shaped vesicles of ~100nm diameter that are initially formed within the endosomal compartment and are secreted when a multivesicular endosome (MVE) fuses with the plasma membrane. It is proposed to determine if exosomes are involved in radiation induced bystander signaling and if so, to investigate the endosomal cargo. Bystander responses have many implications not only for mechanistic understanding of radiation action and its carcinogenic potential but also for radiation risk assessment and the identification of mechanisms that offer potential for prophylactic or therapeutic intervention.

### Methodology:

- (i) Culture of human keratinocyte cell line, HaCaT cells
- (ii) Radiation exposures using the Coblat 60 teletherapy unit at St Luke's Hospital.
- (iii) Harvesting of irradiated cell culture medium and transfer to unirradiated cells
- (iv) Assessment of bystander responses using cell viability assay
- (v) Exosome purification using ultracentrifugation
- (vi) Exosome analysis using fluorescence assays and SDS-PAGE analysis of any proteins associated with exosomal fractions from ICCM

### Learning Outcomes:

- Understanding of non-targeted effects of radiation and the new paradigm in radiation biology
- Experience in cell culture and radiobiological methods
- Experience with data management and statistical analysis
- Experience with report writing, presentations and poster preparation

### Relevance to research programme:

The RESC has an international reputation and considerable experience in non-targeted effects of ionising radiation. This project will build on our previous work on signalling pathways in bystander cells.



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