
Carmel Mothersill
Colin Seymour
McMaster University
Outline

• Bystander effects and genomic instability
• In vivo relevance and evidence
• The fish model
• Mechanisms
• Multiple stressor issues
• Why do these effects happen?
Take home messages

- Target theory, LNT and DNA centric ideas of low dose effects are dead
- Chronic (background) exposure to radiation is in no way related, even by elaborate “fudge factors” to acute exposure
- Environmental effects are not simple and multiple stressor exposure HAS to be part of the discussion
- Evolution is all about adaptation (or not?) to changed conditions
The bystander effect

Ionizing radiation, UVA, UVB, ELF-EMF and heavy metals induce affected cell to signal to others. Responses to the signals include apoptosis, micronucleus formation, transformation, mutation, induction of stress and adaptive pathways. Serotonin (5HT) and Calcium ions known to be involved in signal production.
Bystander and direct dose survival curves over six orders of magnitude $^{60}$Co with calcium data.
The link between bystander effects and genomic instability – twin pillars of the new paradigm

**Old view - clonal outcome**

Progeny are all **clonal** i.e. identical and mutation is passed to all progeny

**New view - non-clonal, population-determined outcome**

Progeny cells are non-clonal and may give rise to a variety of mutations or die

Cells continue to be produced with non-clonal changes
In vivo relevance
In vivo evidence

- Clastogenic factors in blood of irradiated people and experimental animals
- Abscopal effects in distant organs
- Bone marrow ablated mice receiving opposite sex marrow transplant show instability in the regenerating marrow
- Soluble factors from explanted tissues after in vivo exposure
The fish model for studying bystander effects
Measuring radiation induced bystander response in vivo

Irradiate fish
± Metals in water

Dissect fresh tissue from animal
Do proteomics

Chop tissue to provide explant pieces

Culture of explants

Harvest culture medium containing stress signal molecules/metabolomics

Examine explant outgrowth/immunocytochemistry

Add to unirradiated clonogenic cell line and determine survival or other stress endpoints
INDUCTION OF THE BYSTANDER EFFECT IN A DIFFERENT FISH

X-rayed fish

Waterborne bystander effect

Partner bystander effect
Bystander effect induced in vivo in rainbow trout, medaka & zebrafish

0.5Gy X-ray dose → Water soluble bystander signals → Non X-rayed fish

Rainbow trout (Mothersill et al. 2006)

0.5Gy X-ray dose

Zebrafish (Mothersill et al. 2007)

Medaka (Mothersill et al. 2009)
X-RAY AND BYSTANDER EFFECT INDUCED CHANGES TO THE TROUT GILL PROTEOME

SCAF = SR-like CTD-associated factor (SR = Serine – arginine Rich, CTD = C Terminal Domain)
Medaka gill proteins affected

- Complement component C3
- Warm temperature acclimation related 65 kDa protein (Wap65)
- Chromosome 5 SCAF protein
- Creatine kinase
- Lactate dehydrogenase
- Annexin A1
- Annexin A4
- ABC transporter

Molecular size (kDa)

pI (pH units)
Bystander effect proteomics

- Protective response against reactive oxygen species
- Bystander effect proteomic changes are transcriptionally regulated (SCAF proteins)
  - Virtually identical proteomic response in rainbow trout and medaka

Is the bystander effect...

... an immediate protective response (Smith et al 2007) or an adaptation to possible future radiation damage (Kadhim et al 2004)?

- Evidence from trout and medaka suggests the bystander effect is immediately protective
- Additionally the induction of an adaptive response may be species specific and apply particularly in radiosensitive biological systems
Legacy effect of single acute 0.5Gy X-ray exposure to eggs
Transgenerational study (in progress)
Transgenerational memory of irradiation calcium signal

Sham

Sample # 28

Irradiated YSL stage parents

Sample # 1

Bystander to above parents

Sample # 11
Some mechanistic questions

- Role of DNA repair
- Role of serotonin
- Role of p53
DNA repair is important in vivo and in vitro.

Reduced reproductive survival in vitro

DNA repair deficient cell lines and transgenic medaka both produce highly toxic bystander signals after low dose irradiation.

Increased apoptosis in vivo
Serotonin important in vivo and in vitro

Serotonin bound by irradiated cells in vitro, leading to Calcium pulse.

Reserpine inhibits serotonin binding and prevents the bystander effect in vitro and in vivo.
Role of p53 in response to signal but not in generation of signal
Multiple stressors

- Radiation seldom acts alone
- Isotopes are by definition a radioactive chemical
- Little information on synergistic effects
- Little low dose information
- Little in vivo information
What the multiple stressor problem does to radiation protection

- Multiple inducers of stress effects therefore dose and effect are not simply linked
- Response based approach needed
- How to link biological effect with adverse outcome at the organism, population and ecosystem level
- Mechanistic uncertainty at low doses
- Non-targeted effect predominate at low doses
Examples of complex scenarios

- Radiation induces a cell to undergo apoptosis, removing it from the potentially carcinogenic pool. Substance 2 (e.g., Cd) interferes with the signaling cascade and the cell lives – survival assay suggests protective effect of interaction?
- Radiation induces an adaptive response in population A, a further stressor has little effect but pristine population B has no adaption and is devastated by the same stressor.
Fish irradiation in Norway: (let's get some low dose data!)

- Exposure of fish in aerated tanks to mGy doses over 5-48 hours

Metals in the water
Comparison of in vivo mGy radiation exposure ± metals on production of bystander signals
Proposed dose response relationship for radiation in the context of non-targeted effects

Purple arrows indicate mechanistic break points where new, more appropriate, response pathways emerge

Blue line represents old LNT model

Zone of “linearity”

Zone of uncertainty

New “coping” mechanisms induced

Dose-Response 2010
Unifying Theory: transduction of bystander effect

Serotonin (nM range) binds - Activation of calcium channel

Low dose stimulation of system

Activated system

NOTES: “good” response at one level in system may be “bad” at another level. Links stress even mental stress, physiological response and outcome.
So why do these effects happen?
WHY ARE THESE MECHANISMS SO WIDESPREAD AND PERSISTENT?

• In terms of evolution there is conservation of the mechanism and bystander pathway across species and this suggests a very primitive origin in the vertebrates since teleost fish split from the main vertebrate line early in vertebrate evolution.
Population based response?

• Are non-targeted effects a reflection of population level regulation to optimise population fitness (tissue or individual level)?

• Is the function of radiation-induced bystander signaling to co-ordinate behaviour at higher hierarchical levels of organisation?

• Quorum sensing in bacteria is an example of this at the population level as are hormones at the organism level
SUMMARY

Non targeted effects exist
They manifest at high frequency in many ways
They cause “stress-like” symptoms
We know a lot about the mechanisms but little about the reasons why they are tolerated
The underlying debate about purpose or chance is as old as Plato and Aristotle
Acknowledgements

- Thanks to Colin Seymour, husband and long time collaborator
- Richard Smith and all the Salbu Lab
- NOTE EU IP collaborators
- Funded by The Canada Research Chairs and NSERC IRC Chairs programmes