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Emerging Issues in Radiation Protection of Biota- The Impact of Non-Targeted Radiobiological Effects

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Abstract

Ever since the acceptance that non-targeted effects (NTE) can be measured in unirradiated cells or distant progeny of irradiated cells, the discussion has developed about the relevance of these effects for radiation protection, particularly of non-human biota since they increase the complexity of the radiation response and allow for outcomes which are not predictable. For the purposes of this paper, NTE are defined as effects not associated with DNA lesions due to energy deposition in the cell showing the effect and so include genomic instability and bystander effects. Specific examples which will be presented are (i) data showing that bystander mechanisms are either on or off and that the "on" threshold appears to be at a very low dose (mGy range), (ii) data suggesting that adaptive responses are induced not only in neighbouring cells but in organisms which receive bystander signals and (iii) data showing that chronic exposures to alpha or gamma irradiation lead to complex responses in organisms which can be adaptive and protective, (iv) evidence that mixed contaminant exposures which include radiation can have sub-additive or synergistic effects. NTE may call into question radiation effects paradigms such as the linear-non-threshold model (LNT), but may also have relevance to wider mechanisms in biology, concerning process of selection, the transmission of heritable traits, the relevance of "social" interactions between cells, organisms and populations and the mechanism by which cells/organisms respond rapidly to environmental stress. This presentation will also argue that a key consequence of findings in NTE biology is that at any given level of organization, from gene to ecosystem – communication of stress signals and heritability of stress adaptations provide the bridges linking one hierarchical level to the next and enable the rapid propagation of change triggered by stress at one level, resulting in change at a different level.

Introduction

Over a ten year period from 1986-1996, the dominant idea in radiation biology and the basis of radiation protection i.e. that all radiation damage resulted in hit cells from energy deposition in those cells' DNA was finally challenged by four key lines of evidence. First in 1986 our group published a paper saying that de novo appearance of lethal mutations could occur in cells which had "recovered" from irradiation and successfully divided for several generations (1). Second, delayed appearance of de novo non-clonal chromosome aberrations was demonstrated in bone marrow stem cell lineages derived from irradiated stem cells (2). These non-clonal aberrations could not have been introduced at the time of irradiation. Third, a very low dose exposure to alpha radiation resulted in more cells showing chromosome damage than could have been hit by the ionizing particles (3) and forth, medium from irradiated cells was found to cause similar levels of clonogenic cell death and genomic instability as direct irradiation (4,5). Taken together, these papers started the scientific revolution establishing a new paradigm in low dose radiobiology which now is accepted by most radiation biologists but still not understood. The first two papers revealed that genetic change could occur in distant descendants of irradiated progenitor cells after multiple normal successful divisions (genomic instability) while the latter three papers established that genetic change could be induced in cells which were not affected by the mutagen (ionizing radiation) but were in receipt of signals from the irradiated cells (bystander effect). The latter effect has since been established to occur between organisms (6-8) so that unirradiated fish, frogs, mice or plants can pick up signals from irradiated partners and show induced genetic and epigenetic effects. This paper will highlight some of the issues and controversies and discuss implications for radiation protection in general with a specific focus on the new approaches to protection of non-human species.

The range of endpoints seen in directly irradiated cells, their distant descendants and there neighbours are shown in table 1. Clearly similar effects are induced. This has led to concerns that low dose irradiation may be more dangerous than previously thought but an alternative view is that radiation like any stressor induces homeostatic processes aimed at tissue and organism survival. Endpoints measured in individual cells may therefore be misleading. This paper will discuss four areas which are important in radiation protection – particularly of non-human biota where NTE may need to be considered.

Table 1

Comparison of endpoints of damage or change in directly irradiated cells, bystander cells and progeny of directly irradiated cells

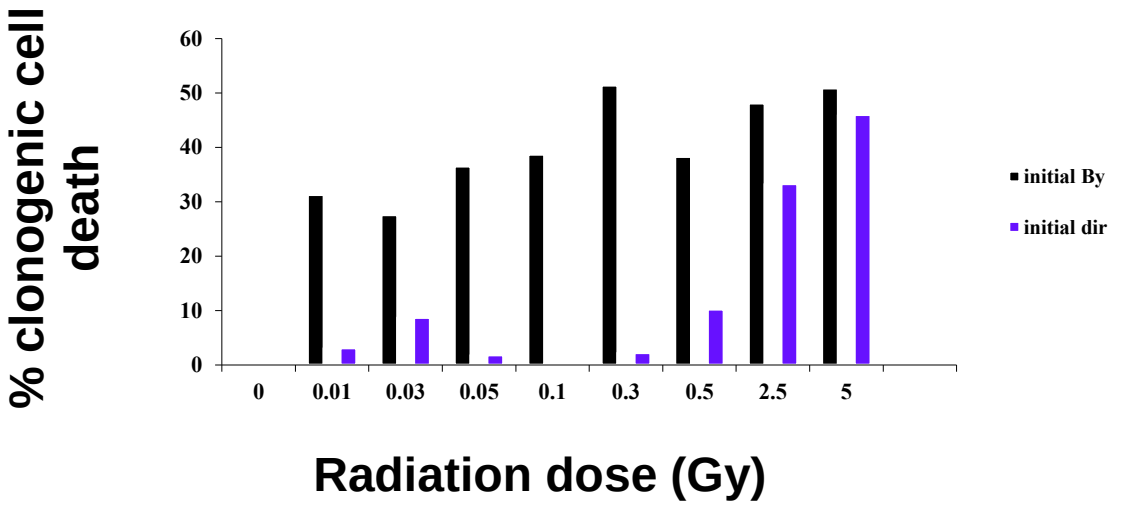
Endpoint	**Directly irradiated cells**	**Radiation-induced bystander cells**	**Progeny of directly irradiated or bystander cells**
Death	Reproductive death, apoptosis	Apoptosis and other forms of cell death	Delayed reproductive death, apoptosis
Protein induction	Induction of repair and checkpoint proteins	Induction of early response proteins	Persistent over-expression of stress proteins in progeny
Reactive oxygen species	Generation of free radicals	Oxidative stress	Persistent oxidative stress
Growth stimulation	Adaptive response	Proliferation and adaptive response	Adaptive response
Non-clonal persistent mutations	Chromosomal aberrations	Genomic instability, lethal mutations	Genomic instability in progeny and lethal mutations
Micronucleus (MN) assay	Increased MN	Cytogenetic effects and increased MN	Cytogenetic effects and increased MN
Carcinogenesis	Transformed foci	Transformed foci	Transformation and cancer in vivo
Mitochondrial function	aberrant	aberrant	aberrant
P53 function	critical	Critical to response outcome	Critical to response outcome
Genotype dependent?	yes	yes	yes

Specific cases where non-targeted effects (NTE) may impact radiation protection of non-human biota

1. Bystander mechanisms are either on or off and that the "on" threshold appears to be at a very low dose (mGy range),

Figure 1 shows a typical dose response for cells exposed to bystander signals (9). The low dose threshold has been established to be in the region of 2mGy (10). Similar data were obtained by Schettino et al (11) who also noted binary behaviour in cell populations exposed to signals from microbeam irradiated cells. Some cells responded while others did not and the on/off process appeared to be random. For radiation protection this means that at low doses between 2mGy and about 500mGy, most of the effect is due to bystander mechanisms. Direct effects do not predominate in the low dose region. This raises important issues about potential protective strategies which will be different when applied after low dose exposure. It may also argue for a low dose threshold for acute exposure below which yet other homeostatic mechanisms may apply.

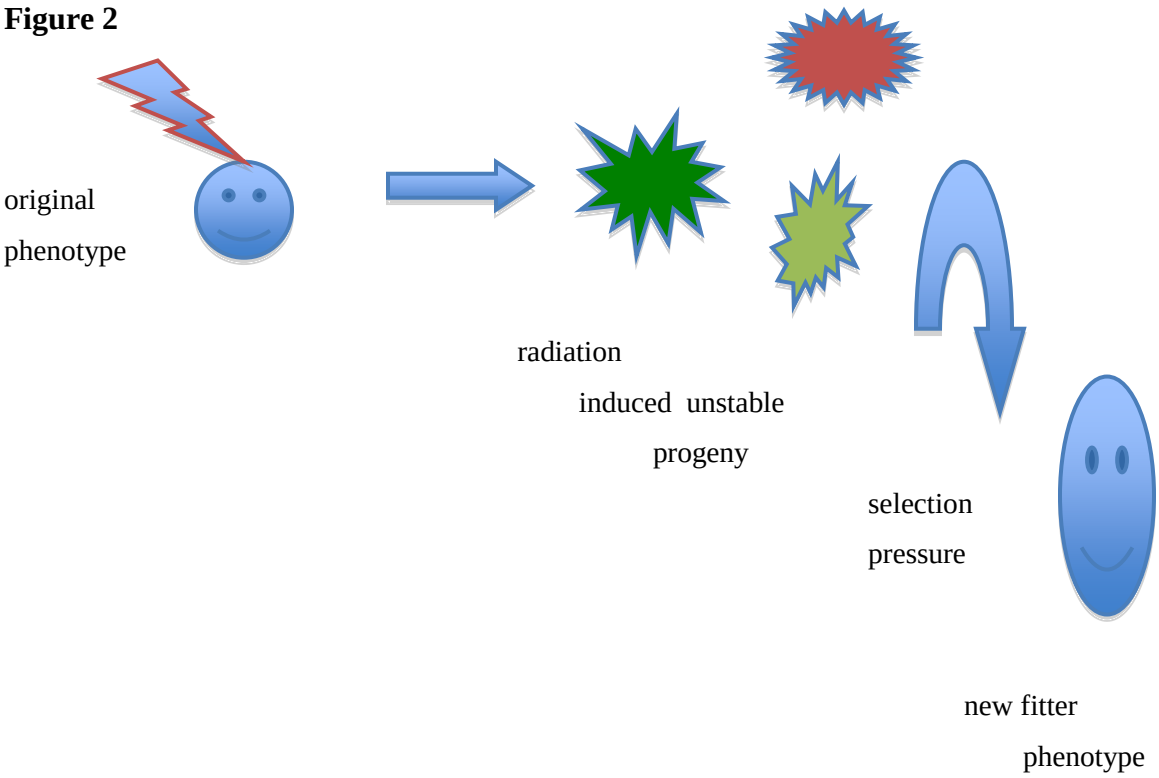
FIGURE 1: Dose response curve showing the separate relative contributions of bystander effect cell death (By) and direct cell death (Dir).



2. Adaptive responses are induced not only in neighbouring cells but in organisms which receive bystander signals

The issue of adaptive response is not usually considered in radiation protection because in situations of uncertainty, the precautionary principle is used. However adaptive responses in *populations* exposed to stressors are not only universal but are a suspected mechanism of evolutionary change. In response to low level radiation stress, cells upregulate protective signaling pathways which reset tolerance for stressors or otherwise reestablish homeostasis (12). Recently it has been demonstrated that in mice and aquatic vertebrates at least, signaling between organisms occurs as well and unaffected (or not yet affected) individuals upregulate protective responses ahead of stressor impact (13,14). Figure 2 shows how this might work to aid adaptive evolution. In terms of protection policy for the environment, it is clearly important to understand these mechanisms and to capture information which is relevant at the population level rather than relying on individual level endpoints of harm or change.

Figure 2



3. Chronic exposures to alpha or gamma irradiation lead to complex responses in organisms which can be adaptive and protective or appear damaging,

Until very recently, exposure to acute doses of radiation were compared to chronic exposures by using a dose and dose-rate effectiveness factor (DDREF) of 2 (15). However, this approach while supported by high dose data from fractionated experiments, may not truly reflect the low dose data from high background chronic exposure senarios. There is evidence (16-18) that populations of animals and plants living in high background areas have a tolerance which means they are resistant to acute exposures. In human populations with the emphasis on individual survival and prevention of radiation-induced cancer, this is a very controversial subject but in biota where population survival is the critical endpoint, it appears clear that adaptive responses need to be considered. It is also important to realize that the impact of an acute release of radioactivity into a pristine environment with no pre-adaptation, could be much more damaging than the same dose received by an adapted population.

4. Mixed contaminant exposures which include radiation can have sub-additive or synergistic effects.

Everyone accepts that radiation is not usually present in isolation in the environment. However doses of radiation are regulated separately from chemical or other physical stressors. This assumes no interaction between the stressors present in a particular situation. The data however suggest complex interactions including adaptive/hormetic responses and synergistic interactions which can augment adverse effects (19,20). Table 2 below highlights some of the key issues in this area. In relation to development of radiation protection policy for the environment, approaches which consider iso-effect doses of different stressors are a way of adding stressor impacts but these may not capture interactive effects unless careful attention is paid to the underlying chemistry and the biological mechanisms which may be in play in the low dose region of dose response curves.

Conclusions

We conclude that NTE are probably very important in low dose and chronic dose radiobiology. In radiation protection, their impact is at present uncertain and depends to a large extent on what else is happening in the environment. Clearly it is important to understand the mechanisms involved and to explore protective or remedial stratagies which arise from these considerations.

Table 2: Issues in the multiple stressor field

- Multiple stressors are the reality
- Need for a number to regulate to
- Compliance can only be measured if there are dose limits or other quantitative parameters to meet
- Legal proof of causation is problematic
- Non-linear dose response curves abound
- Adaptive responses abound
- Saturation responses are seen
- Multiple stressor "doses" in the chemical world can be added together
- But how can we "add" radiation dose and chemical dose?
- What about adaptation, hormetic or synergistic responses, antagonistic effects?
- Might we need a different number for populations from pristine environments with no stress adaptation

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References

[1] C.B. Seymour, C. Mothersill, T. Alper, High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 50 (1986) 167-179.

[2] M.A. Kadhim, D.A. Macdonald, D.T. Goodhead, S.A. Lorimore, S.J. Marsden, E.G. Wright, Transmission of chromosomal instability after plutonium alpha-particle irradiation, Nature. 335 (1992) 738-740.

[3] H. Nagasawa, J.B. Little, Induction of sister chromatid exchanges by extremely low doses of alpha-particles, Cancer Res. 52 (1992) 6394-6396.

[4] C Mothersill, C Seymour. Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells. Int J Radiat Biol. 71 (1997) 421-427.
[5] C.B. Seymour, C. Mothersill, Delayed expression of lethal mutations and genomic instability in the progeny of human epithelial cells that survived in a bystander-killing environment, Radiat. Oncol. Investig. 5 (1997) 106-110.
[6] B. P. Surinov, Mice with radiation or toxic damage or malignant tumors produce aversive chemosignals repelling intact animals. Dokl Biol Sci. 414 (2007) 199-201.
[7] C. Mothersill, B. Bucking, R.W. Smith, N. Agnihotri, A. Oneill, M. Kilemade, C.B. Seymour, Communication of radiation-induced stress or bystander signals between fish in vivo, Environ. Sci. Technol. 40 (2006) 6859-64.
[8] C. Mothersiil, R.W. Smith, N. Agnihotri, C.B. Seymour, Characterization of a radiation-induced stress response communicated in vivo between zebrafish, Environ. Sci. Technol. 41 (2007) 3382-7.
[9] CB Seymour, C Mothersill. Relative contribution of bystander and targeted cell killing to the low-dose region of the radiation dose-response curve. Radiat Res. 153 (2000) 508-511.
[10] Z. Liu, C.E. Mothersill, F.E. McNeill, F.M. Lyng, S.H. Byun, C.B. Seymour, W.V. Prestwich, A dose threshold for a medium transfer bystander effect for a human skin cell line, Radiat Res. 166 (2006) 19-23.
[11] G. Schettino. M. Folkard, B.D. Michael, K.M. Prise, Low-dose binary behavior of bystander cell killing after microbeam irradiation of a single cell with focused c(k) x rays, Radiat. Res. 163 (2005) 332-6.
[12] M. Ojima H. Eto, N. Ban, M. Kai, Radiation-induced bystander effects induce radioadaptive response by low-dose radiation, Radiat. Prot. Dosimetry, 146 (2011) 276-279.
[13] R.W. Smith, J. Wang, C.P. Bucking, C.E. Mothersill, C.B. Seymour, Evidence for a protective response by the gill proteome of rainbow trout exposed to X-ray induced bystander signals, Proteomics. 7 (2007) 4171-80.
[14] M. Audette-Stuart, Bystander effects in Bullfrog tadpoles, Proc. Int. Cong. Radioecol. and Env. Radioactivity, Hamilton, Canada, 2011 (abstract 703)
[15] AD Wrixon New ICRP recommendations. J Radiol Prot. 28 (2008) 161-168.
[16] T. Hertel-Aas, G. Brunborg A. Jaworska, B. Salbu, DH Oughton.
Effects of different gamma exposure regimes on reproduction in the earthworm Eisenia fetida (Oligochaeta). Sci Total Environ. 412-413 (2011) 138-147.

[17] F Fuma, I Kawaguchi, Y Kubota, S Yoshida, Y Kawabata, GG Polikarpov. Effects of chronic γ-irradiation on the aquatic microbial microcosm: equi-dosimetric comparison with effects of heavy metals. J Environ Radioact. 104 (2012) 81-86.
[18] RW Smith, C Mothersill, T Hinton, CB Seymour. Exposure to low level chronic radiation leads to adaptation to a subsequent acute X-ray dose and communication of modified acute X-ray induced bystander signals in medaka (Japanese rice fish, Oryzias latipes). Int J Radiat Biol. 87 (2011) 1011-1022.
[19] C. Mothersill, B. Salbu, L.S. Heier, H.C. Teien, J. Denbeigh, D. Oughton, B.O. Rosseland, C.B. Seymour, Multiple stressor effects of radiation and metals in salmon (Salmo salar), J Environ.. Radioact. 96 (2007) 20-31.
[20] B. Salbu, , J. Denbeigh, R.W. Smith, L.S. Heier, H.C. Teien, B.O. Rosseland, D. Oughton, C.B. Seymour, C. Mothersill, Environmentally relevant mixed exposures to radiation and heavy metals induce measurable stress responses in Atlantic salmon, Environ. Sci. Technol. 42 (2008) 3441-3446.