 doi:10.20944/preprints201906.0231.v1

Relevance of Non-Targeted Effects for Radiotherapy and Diagnostic Radiology

by

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 doi:10.20944/preprints201906.0231.v1

Abstract

Non-targeted effects (NTE) such as bystander effects or genomic instability have been known for many years but their significance for radiotherapy or medical diagnostic radiology are far from clear. Central to the issue are reported differences in response of normal and tumour tissues to signals from directly irradiated cells. This review will discuss possible mechanisms and implications of these different responses and will then discuss possible new therapeutic avenues suggested by the analysis. Finally, the importance of NTE for diagnostic radiology and nuclear medicine which stems from the dominance of NTE in the low dose region of the dose response curve will be presented. Areas such as second cancer induction and microenvironment plasticity will be discussed.

Keywords: bystander effect, genomic instability, lethal mutations,radiotherapy, diagnostic radiology

 doi:10.20944/preprints201906.0231.v1

Non-targeted effects (NTE) in tumours and tumour cell lines are different to those seen normal tissues

a. Review of evidence

Soon after the first publications in recent times relating to lethal mutations, genomic instability, delayed death or bystander effects, now known collectively as non-targeted effects or NTE [1–6], there was controversy in the literature and many reports of failure to find these unexpected consequences in non-irradiated cells or progeny of irradiated cells [7–9]. The initial confusion resolved somewhat when it was realised that all cell types did not show these effects [10,11] and that experimental conditions needed to be carefully controlled [12]. Later papers showed how complex the generation of NTE is with roles for p53, [13–16] serotonin [17–21], TGFβ [22–24], ROS [25–27], cell cycle phase [28,29] and many other factors (for reviews, see [30,31]). However a broad division could be made depending on whether the cell line derived from a p53 mutant or null tumour cell like with low dose radioresistance (a wide shoulder / high α/β ratio) where NTE, at least in the form of bystander effects (BE), were not seen and p53 wild type, low dose radiosensitive cell lines (small shoulder, low α/β ratio) where NTE were pronounced [32]. The particular situation of low dose hypersensitivity/induced radioresistance (HRS/IRR) was particularly interesting since it seemed to be an anomaly until it was found that the BE were expressed in the HRS part of the dose

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response curve but not after the dose response became IRR at higher doses [33,34]. These findings led to the generalisation that fast growing cell lines which demonstrated radioresistance in the low dose range such as HT29 or PC3 [34] were less likely to show BE than slower growing radiosensitivive cells such as HaCaT or SW48 [34]. Later studies with tissues confirmed that tumour derived explants and tumour bearing animals or animals with tumour susceptibility had less pronounced BE than normal tissues or animals [35–38]. The situation with genomic instability (GI) was less clear mainly because endpoints for GI included events resulting in increased death of progeny, such as lethal mutation or delayed death frequency [30,31] or gastrotrichus [39] but also included chromosomal instability endpoints where viable progeny were produced with increased potential for cancer development or transformation in vitro [40,41].

b. Possible reasons/mechanisms

Once the phenomenology of NTE became more fully documented, attention turned to the mechanisms which could allow non-irradiated cells and distant progeny of recovered irradiated cells to display essentially the same endpoints (see table 1). Initially there was a focus on the use of separation techniques such as HPLC to try to determine the size and nature of the proposed molecule which caused NTE. These approaches proved unsuccessful. Attention turned to the response pathways which became the focus of mechanistic studies [42]. Cytokine activation was identified [43] and stress pathways downstream of ROS elevation were documented [44,45].

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Apoptosis was found to be increased in the non-targeted cells [46–49], as were steps in the apoptotic pathway such as calcium flux, mitochondrial membrane depolarisation, caspase 3 release etc. [50–52]. DNA repair proficiency was also shown to be important with radiosensitive mutants (irrespective of the precise mutation) releasing stronger bystander signals than the wild type parents [53–57]. This was later also shown in fish [58]. However, the nature of the signal from the irradiated cells remained a mystery. Breakthroughs came on two aspects of signal production in 2012 when it was shown that exosomes were released by irradiated cells into the culture medium [59,60]. Extracellular vesicles had been suggested early on as vehicles for bystander signals [61] but the molecular tools to analyse their contents were not widely available at the time. With the advent of chip technology and advanced proteomics techniques, screening for relevant proteins and miRNAs became possible [62]. The second breakthrough was when it was shown that irradiation of organic matter (shells, fruits or cells) led to biophoton emission in the UVA range and that there seemed to be a physical component to the initial bystander signal [63–66]. A series of papers [67–69] linked the photon emissions to the extent of the BE and implicated both p53 and exosomes in the mechanism. (see Figure 1). The definitive experiments isolated exosomes and showed that exosomes from cells which received the UVA signal from irradiated cells without medium transfer, could by themselves produce a BE in never irradiated cells [69]. UVA alone was already known to produce BE from research in the 1990's [70,71]. Another piece of the puzzle fell into place with the linking of the UVA biophotons to a block of the activity of mitochondrial complex 1 [72]. This

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leads to depletion of cellular ATP levels and is the type of global issue that could explain many of the reported consequences of low dose radiation exposure such as fatigue, reduced repair capacity and immune system compromise since these are all dependent of cellular energy availability [73,74]. Complex one block has been associated in the literature with UVA exposure and ROS elevation [75]. Currently the race is on in many laboratories to profile exosomes in an attempt to further understand the mechanisms of transmission of bystander signals although the evidence referenced above suggests that biophotons may be sufficient by themselves to induce both BE and GI. Regarding GI the consensus is that this is driven at least in part by BE because GI can be triggered by bystander signals as well as by direct irradiation [76] and harvest of media from descendants of irradiated cells shows perpetuation of signal production in bystander cells [77].

c. Discussion of relevance of smoking and other lifestyle factors

In addition to studying mechanisms associated with cellular genetic type, many studies have been done to look at the effects of environmental and lifestyle factors on the induction of NTE by radiation. These include the effects of heavy metals, organic pollutants, radium and tritium contamination, which confirm that both bystander signalling and GI can be modulated (usually increased) by concomitant exposure to a second stressor. In humans the smoking history and in vitro treatment of human cells and explants with smoking specific nitrosamines prior to irradiation have been studied using a human explant model [78,79]. Radiation-induced BE was

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less toxic in terms of apoptosis induction in explanted tissues from smokers but that was associated with induction of anti-apoptotic proteins and suggests a pro-carcinogenesis rather than pro-apoptosis response to radiation in urothelium from smokers. Treatment of bladder urothelium from non-smokers with the specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), found in the urine of smokers, also induced this phenotype [80].

2. Relevance for therapy: - possible approaches to enhance the therapeutic ratio

a. Inhibition of NTE pathways in normal tissue

The paradox of NTE is that what may be good for an individual cell (e.g. not dying from the radiation dose) may be bad at the level of the population of cells or the organism, if living means carrying potentially carcinogenic damage. Also given that NTE are more prevalent in normal cells than in tumour cells, the adverse effects of NTE in radiotherapy could be considerable. This area has been considered in detail by those studying "out of field" effects [81,82]. Reducing NTE in normal tissue is considered to be a potential novel target for improving radiotherapy outcomes. Those working on the complex one block in mitochondria which is implicated in many non-radiation associated conditions such as chronic fatigue syndrome (CFS) have identified a number of places in the complex where specific activators or

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inhibitors of complex one could be targeted [83]. However the situation may not be as simple as that because there are many reports of NTE induced adaptive and protective responses acting at the population level [84,85] and NTE are seen my some as a mechanism for coordinating normal tissue level response to harmful stimuli such as ionising radiation [86–88]. Clearly it would not be wise to block such a mechanism.

b. Stimulation of NTE pathways in tumour tissues

The corollary of blocking NTE in normal tissues would be to try to stimulate NTE in tumour cell populations. To our knowledge this approach has not been tried but could perhaps involve UVA/biophoton exposure concomitant with radiotherapy, or exposure to antioxidants during therapy to stimulate mitochondrial function. Possibly if exosomes and their specific cargos could be harnessed they could activate NTE in tumours as well.

3. Relevance for diagnostic radiology

Relevance of low dose dominance of NTE

There are two obvious areas of interest here given the enormous increase in the use of radiation-associated techniques in diagnosis of disease [89]. There is

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considerable controversy about whether any harm is being caused by such tests (for reviews see [90–92]). The NTE related concerns relate to the fact that NTE dominate the dose response at low doses and can triggered by acute exposures as low as 2-3mGy and increase until the NTE response saturates at about 0.5Gy at least in vitro [93–95]. There is no information about saturation or initiation doses in vivo or in humans but very early work by this group correlated low dose radiosensitivity (then reported in terms of survival curve shoulder width or n value) with the burden of delayed lethal mutations [96–98]. This suggests that a retrospective analysis of human derived cell lines, or samples where bystander signal information could be obtained, for example the human skin series [99] or the RERF (Radiation Effects research Foundation) blood samples from A-bomb survivors [100] might be interesting to correlate with the subsequent epidemiology. The two main NTE concerns are 2nd cancer induction, and microenvironmental plasticity due to genomic instability.

a. 2nd cancer induction

Cell transformation in vitro has been shown as an endpoint in non-targeted cells [100,101] and modelling of bystander effect impacts on the cancer induction dose response using linear-non-threshold approaches [102] have suggested that NTE may increase the chances of second cancer induction. Further evidence for a potential role of NTE in 2nd cancer induction after low dose exposures comes from

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early data in the literature showing persistent expression of clastogenic factors, micronuclei or microsatellite instability in distant progeny of those exposed or in blood of those exposed several years earlier [103–106]. These data have been reviewed several times but not with respect to the possibility of NTE being involved in second cancer induction (for example [31], [107]).

b. Microenvironmental plasticity

This refers to the ability of the microenvironment to change in response to changes in the system [108]. There is considerable interest currently in what is termed "cross-talk" between functional units in organs and support tissues such as endothelium, fibrous tissue and components of blood and endocrine systems [109]. Maintenance of a healthy microenvironment is critical to the control of function and to the abolition of pre-cancerous cells [110]. Induction of NTE signalling probably has multiple roles depending on other factors such as genetic or epigenetic makeup [111], environmental or lifestyle factors [112] or age [113], all of which can modulate the processes of NTE and the outcomes which may ultimately emerge. Key factors in defining the role of NTE in microenvironmental plasticity are the level at which the effects are of concern e.g. cell, organ, individual or population and the time over which adaptive or mal-adaptive instability has operated. The follow-up epidemiological studies on patients who have experienced low dose medical diagnostic exposure should be an important source of information in this regard.

 doi:10.20944/preprints201906.0231.v1

Conclusion

This short review highlights areas in radiotherapy and diagnostic radiology where non-targeted effects of radiation may be important drivers of outcomes. Areas of most concern relate to the low dose induction of genomic instability and to modulation of normal radiation response pathways by altered signalling due to bystander effects. The importance of context (environmental stressors, lifestyle, age and genetic background are also discussed. Clearly these processes could be involved in determining outcomes after radiation exposure (diagnostic or therapeutic) and should probably be further considered in radiation medicine.

Acknowledgements

We acknowledge the following agencies for funding; The National Chronic Fatigue and Immune Deficiency Syndrome (CFIDS) Foundation Inc, The National Science and Engineering Council (NSERC) of Canada, The Canada Research Council (CRC), The Candu Owner's Group (COG) and Bruce Power.

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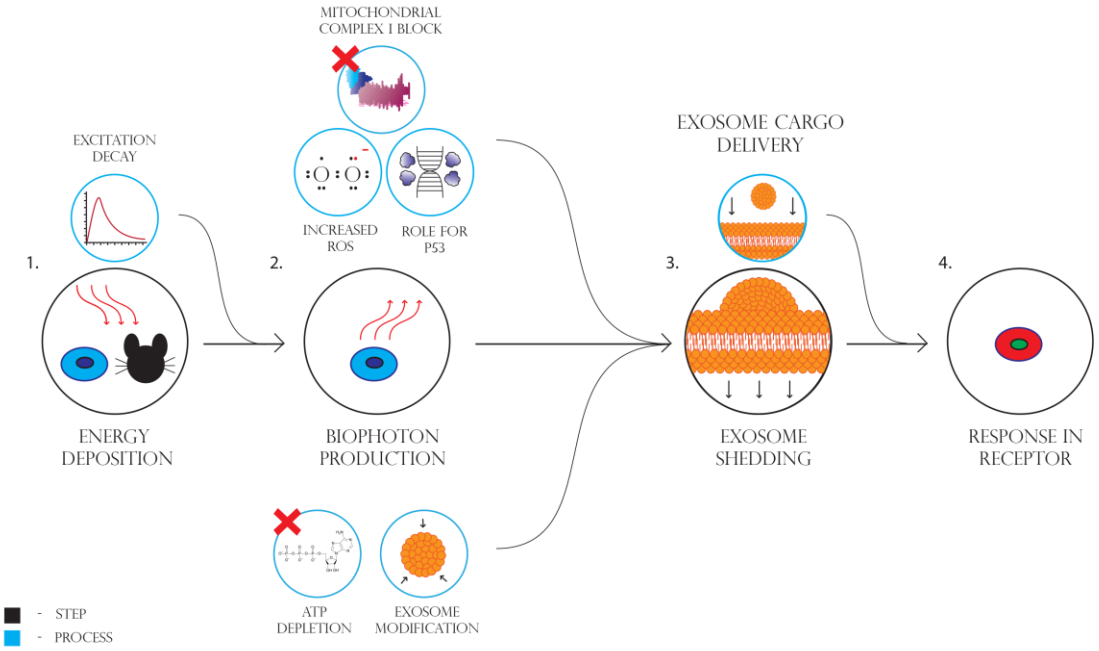
Figure 1: steps and processes involved in the bystander effect as currently understood



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REPORTED EFFECTS

Direct Irradiation Effects	**Effects in Descendant Progeny and Neighbours***
Death	Death
Reproductive Failure	Reproductive Failure
Cellular Apoptosis	Cellular Apoptosis
Mitochondrial Defects	Mitochondrial Defects
Proteomic Changes	Proteomic Changes
Signaling Defects	Signaling Defects
Adaptive Responses	Adaptive Responses
Genetic Differences in Radiosensitivity	Genetic Differences in Radiosensitivity

*Persistent effects in descendant progeny that occur following no further irradiation and in cells neighbouring directly irradiated cells however never directly exposed themselves.

Table 1

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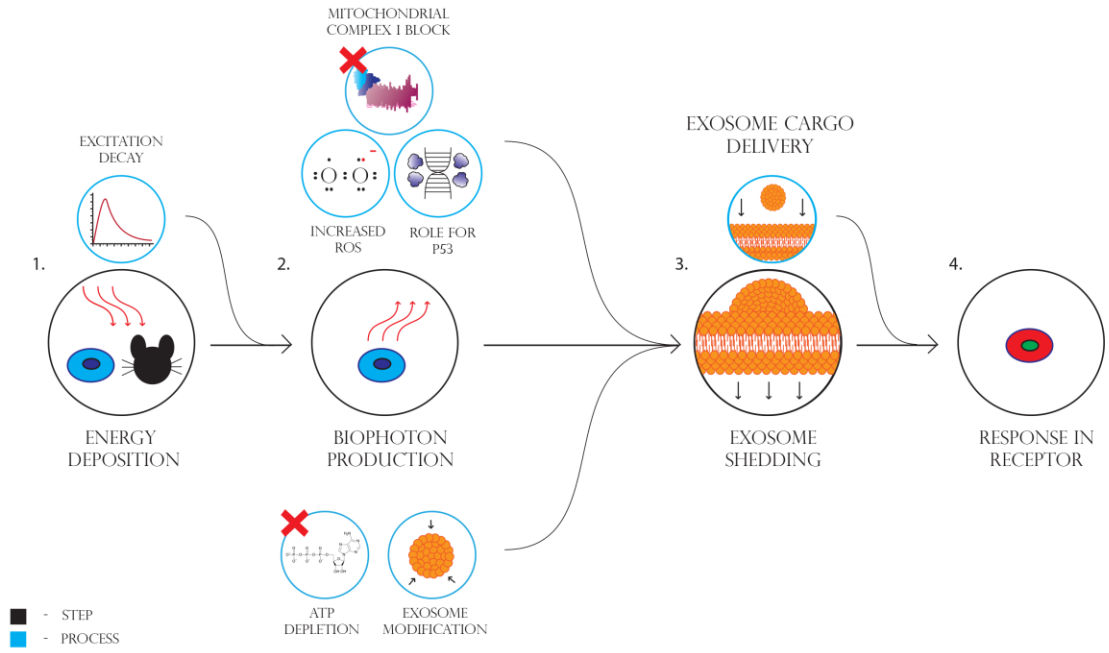

Figure 1

References

1. Seymour, C.B.; Mothersill, C.E.; Alper, T. High Yields of Lethal Mutations in Somatic Mammalian-Cells that Survive Ionizing-Radiation. *Int. J. Radiat. Biol.* **1986**, *50*, 167–179.

2. Mothersill, C.; Seymour, C. Survival of human epithelial cells irradiated with cobalt 60 as microcolonies or single cells. *Int. J. Radiat. Biol.* **1997**, *72*, 597–606.

3. Pampfer, S.; Streffer, C. Increased Chromosome Aberration Levels in Cells from Mouse Fetuses after Zygote X-irradiation. *Int. J. Radiat. Biol.* **1989**, *55*, 85–92.

4. Mothersill, C.; Seymour, C. Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells. *Int. J. Radiat. Biol.* **1997**, *71*, 421–427.

5. Kadhim, M.A.; Macdonald, D.A.; Goodhead, D.T.; Lorimore, S.A.; Marsden, S.J.; Wright, E.G. Transmission of chromosomal instability after plutonium α-particle irradiation. *Nature* **1992**, *355*, 738–740.

6. Nagasawa, H.; Little, J.B. Induction of sister chromatid exchanges by extremely low doses of α-particles. *Cancer Res.* **1992**, *52*, 6394–6396.

7. Sowa Resat, M.B.; Morgan, W.F. Radiation-induced genomic instability: A role for secreted soluble factors in communicating the radiation response to non-irradiated cells. *J. Cell. Biochem.* **2004**, *92*, 1013–1019.

8. Born, R.; Trott, K.R. Clonogenicity of the progeny of surviving cells after irradiation. *Int. J. Radiat. Biol.* **1988**, *53*, 319–330.

9. Limoli, C.L.; Ponnaiya, B.; Corcoran, J.J.; Giedzinski, E.; Kaplan, M.I.; Hartmann, A.; Morgan, W.F. Genomic instability induced by high and low LET ionizing radiation. *Adv. Sp. Res.* **2000**, *25*, 2107–2117.

10. Groesser, T.; Cooper, B.; Rydberg, B. Lack of bystander effects from high-LET radiation for early cytogenetic end points. *Radiat. Res.* **2008**, *170*, 794–802.

11. Ryan, L.A.; Seymour, C.B.; Joiner, M.C.; Mothersill, C.E. Radiation-induced adaptive response is not seen in cell lines showing a bystander effect but is seen in lines showing HRS/IRR response. *Int. J. Radiat. Biol.* **2009**, *85*, 87–95.

12. Mukherjee, S.; Chakraborty, A. Radiation-induced bystander phenomenon: insight and implications in radiotherapy. *Int. J. Radiat. Biol.* **2019**, *95*, 243–263.

13. Strigari, L.; Mancuso, M.; Ubertini, V.; Soriani, A.; Giardullo, P.; Benassi, M.; D'Alessio, D.; Leonardi, S.; Soddu, S.; Bossi, G. Abscopal effect of radiation therapy: Interplay between radiation dose and p53 status. *Int. J. Radiat. Biol.* **2014**, *90*, 248–255.

14. Le, M.; Mothersill, C.E.; Seymour, C.B.; Rainbow, A.J.; McNeill, F.E. An Observed Effect of p53 Status on the Bystander Response to Radiation-Induced Cellular Photon Emission. *Radiat. Res.* **2017**, *187*, 169–185.

15. Colucci, S.; Mothersill, C.; Harney, J.; Gamble, S.C.; Seymour, C.; Arrand, J.E. Induction of multiple PCR-SSCPE mobility shifts in p53 exons in cultures of normal human urothelium exposed to low-dose gamma-radiation. *Int. J. Radiat. Biol.* **1997**, *72*, 21–31.

16. Mothersill, C.; Bristow, R.G.; Harding, S.M.; Smith, R.W.; Mersov, A.; Seymour, C.B. A role for p53 in the response of bystander cells to receipt of medium borne signals from irradiated cells. *Int. J. Radiat. Biol.* **2011**, *87*, 1120–1125.

17. Curtis, J.J.; Seymour, C.B.; Mothersill, C.E. Cell Line-Specific Direct Irradiation and Bystander Responses are Influenced by Fetal Bovine Serum Serotonin Concentrations. *Radiat. Res.* **2018**.

18. Kalanxhi, E.; Dahle, J. The role of serotonin and p53 status in the radiation-induced bystander effect. *Int. J. Radiat. Biol.* **2012**, *88*, 773–776.

19. Poon, R.C.C.; Agnihotri, N.; Seymour, C.; Mothersill, C. Bystander effects of ionizing radiation can be modulated by signaling amines. *Environ. Res.* **2007**, *105*, 200–211.

20. Mothersill, C.; Antonelli, F.; Dahle, J.; Dini, V.; Hegyesi, H.; Iliakis, G.; Kamarainen, K.; Launonen, V.; Lumniczky, K.; Lyng, F.; et al. A laboratory inter-comparison of the importance of serum serotonin levels in the measurement of a range of radiation-induced bystander effects: Overview of study and results presentation. *Int. J. Radiat. Biol.* **2012**, *88*, 763–769.

21. Klammer, H.; Iliakis, G. The impact of serotonin on the development of bystander damage assessed by γ-H2AX foci analysis. *Int. J. Radiat. Biol.* **2012**, *88*, 777–780.

22. Gow, M.D.; Seymour, C.B.; Ryan, L. a; Mothersill, C.E. Induction of bystander response in human glioma cells using high-energy electrons: a role for TGF-beta1. *Radiat. Res.* **2010**, *173*, 769–778.

23. Yin, X.; Tian, W.; Wang, L.; Wang, J.; Zhang, S.; Cao, J.; Yang, H. Radiation quality-dependence of bystander effect in unirradiated fibroblasts is associated with TGF-β1-Smad2 pathway and miR-21 in irradiated keratinocytes. *Sci. Rep.* **2015**, *5*, 11373.

24. Hu, W.; Xu, S.; Yao, B.; Hong, M.; Wu, X.; Pei, H.; Chang, L.; Ding, N.; Gao, X.; Ye, C. MiR-663 inhibits radiation-induced bystander effects by targeting TGFB1 in a feedback mode. *RNA Biol.* **2014**, *11*, 1189–1198.

25. Lyng, F.M.; Seymour, C.B.; Mothersill, C. Oxidative stress in cells exposed to low levels of ionizing radiation. *Biochem. Soc. Trans.* **2001**, *29*, 350–353.

26. Clutton, S.M.; Townsend, K.M.S.; Walker, C.; Ansell, J.D.; Wright, E.G. Radiation-induced genomic instability and persisting oxidative stress in primary bone marrow cultures. *Carcinogenesis* **1996**, *17*, 1633–1639.

27. Sharma, N.; Colangelo, N.W.; de Toledo, S.M.; Azzam, E.I. Diffusible factors secreted by glioblastoma and medulloblastoma cells induce oxidative stress in bystander neural stem progenitors. *ASN Neuro* **2016**, *8*, 1759091416662808.

28. Tu, W.; Dong, C.; Konishi, T.; Kobayashi, A.; Furusawa, Y.; Uchihori, Y.; Xie, Y.; Dang, B.; Li, W.; Shao, C. G2-M phase-correlative bystander effects are co-mediated by DNA-PKcs and ATM after carbon ion irradiation. *Mutat. Res. Toxicol. Environ. Mutagen.* **2016**, *795*, 1–6.

29. Yang, S.; Xu, J.; Shao, W.; Geng, C.; Li, J.; Guo, F.; Miao, H.; Shen, W.; Ye, T.; Liu, Y. Radiation-induced bystander effects in A549 cells exposed to 6 MV X-rays. *Cell Biochem. Biophys.* **2015**, *72*, 877–882.

30. Burdak-Rothkamm, S.; Rothkamm, K. Radiation-induced bystander and

systemic effects serve as a unifying model system for genotoxic stress responses. *Mutat. Res. Mutat. Res.* **2018**.

31. Mothersill, C.; Rusin, A.; Fernandez-Palomo, C.; Seymour, C. History of bystander effects research 1905-present; what is in a name? *Int. J. Radiat. Biol.* **2018**, *94*, 696–707.

32. Joiner, M.C.; Marples, B.; Lambin, P.; Short, S.C.; Turesson, I. Low-dose hypersensitivity: current status and possible mechanisms. *Int. J. Radiat. Oncol. Biol. Phys.* **2001**, *49*, 379–389.

33. Fernandez-Palomo, C.; Seymour, C.; Mothersill, C. Inter-Relationship between Low-Dose Hyper-Radiosensitivity and Radiation-Induced Bystander Effects in the Human T98G Glioma and the Epithelial HaCaT Cell Line. *Radiat. Res.* **2016**, *185*, 124–33.

34. Mothersill, C.; Seymour, C.B.; Joiner, M.C.; May, N.; Mothersill, C.; Seymoura, I.C.B.; Joinerc, M.C. Relationship between Radiation-Induced Low-Dose Hypersensitivity and the Bystander Effect. *Radiat. Res.* **2002**, *157*, 526–532.

35. Mothersill, C.; Lyng, F.; OReilly, S.; Harney, J.; Seymour, C.B. Expression of lethal mutations is suppressed in neoplastically transformed cells and after treatment of normal cells with carcinogens. *Radiat. Res.* **1996**, *145*, 714–721.

36. Mothersill, C.E.; O'Malley, K.J.; Murphy, D.M.; Seymour, C.B.; Lorimore, S.A.; Wright, E.G. Identification and characterization of three subtypes of radiation response in normal human urothelial cultures exposed to ionizing radiation. *Carcinogenesis* **1999**, *20*, 2273–2278.

37. Fernandez-Palomo, C.; Schueltke, E.; Smith, R.; Braeuer-Krisch, E.; Laissue, J.; Schroll, C.; Fazzari, J.; Seymour, C.; Mothersill, C. Bystander effects in tumor-free and tumor-bearing rat brains following irradiation by synchrotron X-rays. *Int. J. Radiat. Biol.* **2013**, *89*, 445–453.

38. Mothersill, C.; Lyng, F.; Seymour, C.; Maguire, P.; Lorimore, S.; Wright, E. Genetic factors influencing bystander signaling in murine bladder epithelium after low-dose irradiation in vivo. *Radiat. Res.* **2005**, *163*, 391–399.

39. Pampfer, S.; Streffer, C.; Müller, W.-U. Micronucleus formation in 2-cell embryos after in vitro X-irradiation of mouse spermatozoa. *Mutat. Res. Mol. Mech. Mutagen.* **1989**, *210*, 191–196.

40. Burtt, J.J.; Thompson, P.A.; Lafrenie, R.M. Non-targeted effects and radiation-induced carcinogenesis: a review. *J. Radiol. Prot.* **2016**, *36*, R23.

41. Yahyapour, R.; Motevaseli, E.; Rezaeyan, A.; Abdollahi, H.; Farhood, B.; Cheki, M.; Najafi, M.; Villa, V. Mechanisms of radiation bystander and non-targeted effects: implications to radiation carcinogenesis and radiotherapy. *Curr. Radiopharm.* **2018**, *11*, 34–45.

42. K Hei, T.; Zhou, H.; Chai, Y.; Ponnaiya, B.; N Ivanov, V. Radiation induced non-targeted response: mechanism and potential clinical implications. *Curr. Mol. Pharmacol.* **2011**, *4*, 96–105.

43. Rodel, F.; Frey, B.; Multhoff, G.; Gaipl, U. Contribution of the immune system to bystander and non-targeted effects of ionizing radiation. *Cancer Lett.* **2015**, *356*, 105–113.

44. Lyng, F.M.; Maguire, P.; McClean, B.; Seymour, C.; Mothersill, C. The involvement of calcium and MAP kinase signaling pathways in the production of radiation-induced bystander effects. *Radiat. Res.* **2006**, *165*, 400–409.

45. Hamada, N.; Maeda, M.; Otsuka, K.; Tomita, M. Signaling pathways underpinning the manifestations of ionizing radiation-induced bystander effects. *Curr. Mol. Pharmacol.* **2011**, *4*, 79–95.

46. Furlong, H.; Mothersill, C.; Lyng, F.M.; Howe, O. Apoptosis is signalled early by low doses of ionising radiation in a radiation-induced bystander effect. *Mutat.*

Res. - Fundam. Mol. Mech. Mutagen. **2013**, *741–742*, 35–43.

47. Sawal, H.A.; Asghar, K.; Bureik, M.; Jalal, N. Bystander signaling via oxidative metabolism. *Onco. Targets. Ther.* **2017**, *10*, 3925–3940.

48. Jella, K.K.; Garcia, A.; McClean, B.; Byrne, H.J.; Lyng, F.M. Cell death pathways in directly irradiated cells and cells exposed to medium from irradiated cells. *Int. J. Radiat. Biol.* **2013**, *89*, 182–190.

49. Kovalchuk, O.; Zemp, F.J.; Filkowski, J.N.; Altamirano, A.M.; Dickey, J.S.; Jenkins-Baker, G.; Marino, S.A.; Brenner, D.J.; Bonner, W.M.; Sedelnikova, O.A. microRNAome changes in bystander three-dimensional human tissue models suggest priming of apoptotic pathways. *Carcinogenesis* **2010**, *31*, 1882–1888.

50. Lyng, F.M.; Seymour, C.B.; Mothersill, C. Production of a signal by irradiated cells which leads to a response in unirradiated cells characteristic of initiation of apoptosis. *Br. J. Cancer* **2000**, *83*, 1223–1230.

51. Shao, C.; Lyng, F.M.; Folkard, M.; Prise, K.M. Calcium fluxes modulate the radiation-induced bystander responses in targeted glioma and fibroblast cells. *Radiat. Res.* **2006**, *166*, 479–487.

52. Lyng, F.M.; Howe, O.L.; McClean, B. Reactive oxygen species-induced release of signalling factors in irradiated cells triggers membrane signalling and calcium influx in bystander cells. *Int. J. Radiat. Biol.* **2011**, *87*, 683–695.

53. Mothersill, C.; Seymour, R.J.; Seymour, C.B. Increased radiosensitivity in cells of two human cell lines treated with bystander medium from irradiated repair-deficient cells. *Radiat. Res.* **2006**, *165*, 26–34.

54. Mothersill, C.; Seymour, R.J.; Seymour, C.B. Bystander effects in repair-deficient cell lines. *Radiat. Res.* **2004**, *161*, 256–263.

55. Zhang, Y.; Zhou, J.; Held, K.D.; Redmond, R.W.; Prise, K.M.; Liber, H.L.

 doi:10.20944/preprints201906.0231.v1

Deficiencies of double-strand break repair factors and effects on mutagenesis in directly gamma-irradiated and medium-mediated bystander human lymphoblastoid cells. *Radiat. Res.* **2008**, *169*, 197–206.

56. Tu, W.; Dong, C.; Fu, J.; Pan, Y.; Kobayashi, A.; Furusawa, Y.; Konishi, T.; Shao, C. Both irradiated and bystander effects link with DNA repair capacity and the linear energy transfer. *Life Sci.* **2019**, *222*, 228–234.

57. Mladenov, E.; Li, F.; Zhang, L.; Klammer, H.; Iliakis, G. Intercellular communication of DNA damage and oxidative status underpin bystander effects. *Int. J. Radiat. Biol.* **2018**, *94*, 719–726.

58. Mothersill, C.; Smith, R.W.; Hinton, T.G.; Aizawa, K.; Seymour, C.B. Communication of Radiation-Induced Signals in Vivo between DNA Repair Deficient and Proficient Medaka (Oryzias latipes). *Environ. Sci. Technol.* **2009**, *43*, 3335–3342.

59. Al-Mayah, A.H.J.; Irons, S.L.; Pink, R.C.; Carter, D.R.F.; Kadhim, M.A. Possible role of exosomes containing RNA in mediating nontargeted effect of ionizing radiation. *Radiat. Res.* **2012**, *177*, 539–545.

60. Jella, K.K.; Rani, S.; O'Driscoll, L.; McClean, B.; Byrne, H.J.; Lyng, F.M. Exosomes are involved in mediating radiation induced bystander signaling in human keratinocyte cells. *Radiat. Res.* **2014**, *181*, 138–145.

61. Albanese, J.; Dainiak, N. Modulation of intercellular communication mediated at the cell surface and on extracellular, plasma membrane-derived vesicles by ionizing radiation. *Exp. Hematol.* **2003**, *31*, 455–464.

62. Yentrapalli, R.; Merl-Pham, J.; Azimzadeh, O.; Mutschelknaus, L.; Peters, C.; Hauck, S.M.; Atkinson, M.J.; Tapio, S.; Moertl, S. Quantitative changes in the protein and miRNA cargo of plasma exosome-like vesicles after exposure to ionizing radiation. *Int. J. Radiat. Biol.* **2017**, *93*, 569–580.

63. Ahmad, S.B.; McNeill, F.E.; Byun, S.H.; Prestwich, W. V.; Mothersill, C.; Seymour, C.; Armstrong, A.; Fernandez, C. Ultra-violet light emission from hpv-g cells irradiated with low let radiation from 90Y; consequences for radiation induced bystander effects. *Dose-Response* **2013**, *11*, 498–516.

64. Ahmad, S.B.; McNeill, F.E.; Byun, S.H.; Prestwich, W. V; Seymour, C.; Mothersill, C.E. Ion beam induced luminescence: Relevance to radiation induced bystander effects. *Nucl. INSTRUMENTS METHODS Phys. Res. Sect. B-BEAM Interact. WITH Mater. ATOMS* **2012**, *288*, 81–88.

65. Mothersill, C.; Moran, G.; McNeill, F.; Gow, M.D.; Denbeigh, J.; Prestwich, W.; Seymour, C.B. A role for bioelectric effects in the induction of bystander signals by ionizing radiation? *Dose-Response* **2007**, *5*, dose-response.

66. Ishii, M.; Rohrer, B. Bystander effects elicited by single-cell photo-oxidative blue-light stimulation in retinal pigment epithelium cell networks. *Cell death Discov.* **2017**, *3*, 16071.

67. Le, M.; McNeill, F.E.; Seymour, C.; Rainbow, A.J.; Mothersill, C.E. An observed effect of ultraviolet radiation emitted from beta-irradiated HaCaT cells upon non-beta-irradiated bystander cells. *Radiat. Res.* **2015**, *183*, 279–90.

68. Le, M.; Mothersill, C.E.; Seymour, C.B.; Ahmad, S.B.; Armstrong, A.; Rainbow, A.J.; McNeill, F.E. Factors affecting ultraviolet-A photon emission from beta-irradiated human keratinocyte cells. *Phys. Med. Biol.* **2015**, *60*, 6371–6389.

69. Le, M.; Fernandez-Palomo, C.; McNeill, F.E.; Seymour, C.B.; Rainbow, A.J.; Mothersill, C.E. Exosomes are released by bystander cells exposed to radiation-induced biophoton signals: Reconciling the mechanisms mediating the bystander effect. *PLoS One* **2017**, *12*, e0173685.

70. Whiteside, J.R.; McMillan, T.J. A bystander effect is induced in human cells treated with UVA radiation but not UVB radiation. *Radiat. Res.* **2009**, *171*, 204–211.

71. O'Reilly, P.; Mothersill, C. Comparative effects of UV A and UV B on clonogenic survival and delayed cell death in skin cell lines from humans and fish. *Int. J. Radiat. Biol.* **1997**, *72*, 111–119.

72. Le, M.; McNeill, F.E.; Seymour, C.B.; Rusin, A.; Diamond, K.; Rainbow, A.J.; Murphy, J.; Mothersill, C.E. Modulation of oxidative phosphorylation (OXPHOS) by radiation- induced biophotons. *Environ. Res.* **2018**, *163*.

73. Rusin, A.; Seymour, C.; Mothersill, C. Chronic Fatigue and Immune Deficiency Syndrome (CFIDS), cellular metabolism, and ionizing radiation: A review of contemporary scientific literature and suggested directions for future research. *Int. J. Radiat. Biol.* **2018**, 1–63.

74. Ryan, J.L.; Carroll, J.K.; Ryan, E.P.; Mustian, K.M.; Fiscella, K.; Morrow, G.R. Mechanisms of cancer-related fatigue. *Oncologist* **2007**, *12 Suppl 1*, 22–34.

75. Fujita, D.; Murai, M.; Nishioka, T.; Miyoshi, H. Light control of mitochondrial complex I activity by a photoresponsive inhibitor. *Biochemistry* **2006**, *45*, 6581–6586.

76. Lorimore, S.A.; Chrystal, J.A.; Robinson, J.I.; Coates, P.J.; Wright, E.G. Chromosomal instability in unirradiated hemaopoietic cells induced by macrophages exposed in vivo to ionizing radiation. *Cancer Res.* **2008**, *68*, 8122–8126.

77. Seymour, C.B.; Mothersill, C. 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.* **1997**, *5*, 106–110.

78. Mothersill, C.; OMalley, K.; Colucci, S.; Murphy, D.; Lynch, T.; Payne, S.; Seymour, C.; Harney, J. p53 protein expression and increased SSCP mobility shifts in the p53 gene in normal urothelium cultured from smokers. *Carcinogenesis* **1997**, *18*, 1241–1245.

79. Colucci, S.; ElGehani, R.; Flint, S.; Mothersill, C. p53 mutations and protein expression in primary cultures of normal oral mucosa in smokers and non-smokers. *ORAL Oncol.* **1997**, *33*, 240–246.

80. Lyng, F.M.; deFeijterRupp, H.L.; Hayashi, T.; OMalley, K.; Murphy, D.M.; Cottell, D.C.; Trosko, J.E.; Seymour, C.B.; Mothersill, C. Effect of a tobacco-related nitrosamine on intercellular communication in human urothelial cells: A possible factor in smoking-related bladder carcinogenesis. *Oncol. Res.* **1996**, *8*, 371–378.

81. Shields, L.; Vega-Carrascal, I.; Singleton, S.; Lyng, F.M.; McClean, B. Cell survival and DNA damage in normal prostate cells irradiated out-of-field. *Radiat. Res.* **2014**, *182*, 499–506.

82. Hanna, G.G.; Coyle, V.M.; Prise, K.M. Immune modulation in advanced radiotherapies: Targeting out-of-field effects. *Cancer Lett.* **2015**, *368*, 246–251.

83. Li, N.; Ragheb, K.; Lawler, G.; Sturgis, J.; Rajwa, B.; Melendez, J.A.; Robinson, J.P. Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J. Biol. Chem.* **2003**, *278*, 8516–8525.

84. Mothersill, C.; Seymour, C. Radiation-induced bystander effects and adaptive responses - the Yin and Yang of low dose radiobiology? *Mutat. Res. Mol. Mech. Mutagen.* **2004**, *568*, 121–128.

85. Sawant, S.G.; Randers-Pehrson, G.; Metting, N.F.; Hall, E.J. Adaptive response and the bystander effect induced by radiation in C3H 10T½ cells in culture. *Radiat. Res.* **2001**, *156*, 177–180.

86. Mothersill, C.; Seymour, C. Eco-systems biology-From the gene to the stream. *Mutat. Res. Mol. Mech. Mutagen.* **2010**, *687*, 63–66.

87. Bewicke-Copley, F.; Mulcahy, L.A.; Jacobs, L.A.; Samuel, P.; Akbar, N.; Pink, R.C.; Carter, D.R.F. Extracellular vesicles released following heat stress induce bystander effect in unstressed populations. *J. Extracell. vesicles* **2017**, *6*, 1340746.

88. Trosko, J.E. Hierarchical and cybernetic nature of biologic systems and their relevance to homeostatic adaptation to low-level exposures to oxidative stress-inducing agents. *Environ. Health Perspect.* **1998**, *106 Suppl 1*, 331–339.

89. Sodhi, K.S.; Krishna, S.; Saxena, A.K.; Sinha, A.; Khandelwal, N.; Lee, E.Y. Clinical application of "Justification" and "Optimization" principle of ALARA in pediatric CT imaging: 'How many children can be protected from unnecessary radiation?'. *Eur. J. Radiol.* **2015**, *84*, 1752–1757.

90. Sheppard, J.P.; Nguyen, T.; Alkhalid, Y.; Beckett, J.S.; Salamon, N.; Yang, I. Risk of Brain Tumor Induction from Pediatric Head CT Procedures: A Systematic Literature Review. *Brain tumor Res. Treat.* **2018**, *6*, 1–7.

91. Meulepas, J.M.; Hauptmann, M.; Lubin, J.H.; Shuryak, I.; Brenner, D.J. Is there Unmeasured Indication Bias in Radiation-Related Cancer Risk Estimates from Studies of Computed Tomography? *Radiat. Res.* **2018**, *189*, 128–135.

92. Malone, J.; Zölzer, F.; Meskens, G.; Skourou, C. *Ethics for Radiation Protection in Medicine*; CRC Press, 2018; ISBN 1351372491.

93. Seymour, C.B.; Mothersill, C. Relative contribution of bystander and targeted cell killing to the low-dose region of the radiation dose-response curve. *Radiat. Res.* **2000**, *153*, 508–511.

94. Liu, Z.F.; Mothersill, C.E.; McNeill, F.E.; Lyng, F.M.; Byun, S.H.; Seymour, C.B.; Prestwich, W. V A dose threshold for a medium transfer bystander effect for a human skin cell line. *Radiat. Res.* **2006**, *166*, 19–23.

95. Schettino, G.; Folkard, M.; Michael, B.D.; Prise, K.M. Low-dose binary behavior

of bystander cell killing after microbeam irradiation of a single cell with focused CK X rays. *Radiat. Res.* **2005**, *163*, 332–336.

96. SEYMOUR, C.B.; MOTHERSILL, C. LETHAL MUTATIONS, THE SURVIVAL-CURVE SHOULDER AND SPLIT-DOSE RECOVERY. *Int. J. Radiat. Biol.* **1989**, *56*, 999–1010.

97. ALPER, T.; MOTHERSILL, C.; SEYMOUR, C.B. LETHAL MUTATIONS ATTRIBUTABLE TO MISREPAIR OF Q-LESIONS. *Int. J. Radiat. Biol.* **1988**, *54*, 525–530.

98. Mothersill, C.; Seymour, C. Targets, pools, shoulders, and communication - a reflection on the evolution of low-dose radiobiology. *Int. J. Radiat. Biol.* **2019**, 1–10.

99. Burnet, N.G.; Nyman, J.; Turesson, I.; Wurm, R.; Yarnold, J.R.; Peacock, J.H. Prediction of normal-tissue tolerance to radiotherapy from in-vitro cellular radiation sensitivity. *Lancet (London, England)* **1992**, *339*, 1570–1571.

100. Hsu, W.-L.; Tatsukawa, Y.; Neriishi, K.; Yamada, M.; Cologne, J.; Fujiwara, S. Longitudinal trends of total white blood cell and differential white blood cell counts of atomic bomb survivors. *J. Radiat. Res.* **2010**, *51*, 431–439.

101. Lewis, D.A.; Mayhugh, B.M.; Qin, Y.; Trott, K.; Mendonca, M.S. Production of delayed death and neoplastic transformation in CGL1 cells by radiation-induced bystander effects. *Radiat. Res.* **2001**, *156*, 251–258.

102. Brenner, D.J.; Little, J.B.; Sachs, R.K. The bystander effect in radiation oncogenesis: II. A quantitative model. *Radiat. Res.* **2001**, *155*, 402–408.

103. Marozik, P.; Mothersill, C.; Seymour, C.B.; Mosse, I.; Melnov, S. Bystander effects induced by serum from survivors of the Chernobyl accident. *Exp. Hematol.* **2007**, *35*, 55–63.

104. Emerit, I.; Quastel, M.; Goldsmith, J.; Merkin, L.; Levy, A.; Cernjavski, L.; Alaoui-Youssefi, A.; Pogossian, A.; Riklis, E. Clastogenic factors in the plasma of children exposed at Chernobyl. *Mutat. Res.* **1997**, *373*, 47–54.

105. Dubrova, Y.E.; Nesterov, V.N.; Krouchinsky, N.G.; Ostapenko, V.A.; Neumann, R.; Neil, D.L.; Jeffreys, A.J. Human minisatellite mutation rate after the Chernobyl accident. *Nature* **1996**, *380*, 683–686.

106. Dubrova, Y.E.; Nesterov, V.N.; Krouchinsky, N.G.; Ostapenko, V.A.; Vergnaud, G.; Giraudeau, F.; Buard, J.; Jeffreys, A.J. Further evidence for elevated human minisatellite mutation rate in Belarus eight years after the Chernobyl accident. *Mutat. Res.* **1997**, *381*, 267–278.

107. Morgan, W.F. Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. *Radiat. Res.* **2003**, *159*, 581–596.

108. Wisdom, K.M.; Adebowale, K.; Chang, J.; Lee, J.Y.; Nam, S.; Desai, R.; Rossen, N.S.; Rafat, M.; West, R.B.; Hodgson, L.; et al. Matrix mechanical plasticity regulates cancer cell migration through confining microenvironments. *Nat. Commun.* **2018**, *9*, 4144.

109. Gandhi, S.; Chandna, S. Radiation-induced inflammatory cascade and its reverberating crosstalks as potential cause of post-radiotherapy second malignancies. *Cancer Metastasis Rev.* **2017**, *36*, 375–393.

110. Lee, G.; Hall, R.R. 3rd; Ahmed, A.U. Cancer Stem Cells: Cellular Plasticity, Niche, and its Clinical Relevance. *J. Stem Cell Res. Ther.* **2016**, *6*.

111. Schofield, P.N.; Kondratowicz, M. Evolving paradigms for the biological response to low dose ionizing radiation; the role of epigenetics. *Int. J. Radiat. Biol.* **2018**, *94*, 769–781.

 doi:10.20944/preprints201906.0231.v1

112. Perduca, V.; Omichessan, H.; Baglietto, L.; Severi, G. Mutational and epigenetic signatures in cancer tissue linked to environmental exposures and lifestyle. *Curr. Opin. Oncol.* **2018**, *30*, 61–67.

113. Liggett, L.A.; DeGregori, J. Changing mutational and adaptive landscapes and the genesis of cancer. *Biochim. Biophys. acta. Rev. cancer* **2017**, *1867*, 84–94.