Systemic effects of synchrotron radiation

To cite this article: J Ventura et al 2019 J. Phys.: Conf. Ser. 1154 012028

View the article online for updates and enhancements.

You may also like
- Evaluating the potential for maximized T cell redistribution entropy to improve abscopal responses to radiotherapy
  Rachel Walker, Jonathan D Schoentfeld, Shari Pilon-Thomas et al.
- Rational nanocarrier design towards clinical translation of cancer nanotherapy
  Dandan Guo, Xiaotian Ji and Juntao Luo
- Enhancing low-dose risk assessment using mechanistic mathematical models of radiation effects
  Igor Shuryak
Systemic effects of synchrotron radiation

J Ventura1,2, C N Sprung3, H B Forrester3, J S Palazzolo1, A Ivashkevich3,4, A W Stevenson5,6, C J Hall6, A G Georgakilas7, P N Lobachevsky1,8 and O A Martin1,8,9
1Research Division, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia
2University of Melbourne Department of Obstetrics & Gynaecology and Royal Women’s Hospital, Melbourne, VIC, Australia
3Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research and Monash University, Clayton, VIC, Australia
4Radiation Oncology, Canberra Hospital, Garran, ACT, Australia
5CSIRO, Clayton, VIC, Australia; 6Australian Synchrotron, Clayton, VIC, Australia
7DNA Damage Laboratory, Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens, Athens, Greece
8Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC, Australia
9Division of Radiation Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

E-mail: olga.martin@petermac.org

Abstract. A change in an organ or tissue distant from the irradiated region was termed the radiation-induced abscopal effect (RIAE). It is not known how radiation settings affect non-targeted normal tissues and therefore the risk of radiation-related adverse abscopal effects. In a recent study, we examined abscopal effects of microbeam radiotherapy (MRT) and broad beam (BB) configurations, in mice that were locally exposed to a very short pulse of a high dose-rate synchrotron beam utilizing the Imaging and Medical Beamline (IMBL) at the Australian Synchrotron. Here we summarise this study. Oxidative DNA damage was elevated in a wide variety of unirradiated normal tissues. Out-of-field duodenum showed a trend for elevated apoptotic cell death under most irradiation conditions, however, double-strand breaks (DSBs) elevated only after exposure to lower doses. These genotoxic events were accompanied by changes in concentrations of several plasma cytokines and in frequencies of macrophages, neutrophils and T-lymphocytes in duodenum. Overall, systemic radiation responses were independent of dose, time post-irradiation, and radiation modality. These findings have implications for the planning of therapeutic and diagnostic radiation treatment to reduce the risk of radiation-related adverse systemic effects.

1. Introduction
The radiation-induced bystander effect (RIBE) is now recognised as a consequence of radiotherapy [1]. Evidence has revealed that irradiated cells not only experience the direct DNA damage effects but also exchange signals with non-irradiated distant or neighbouring cells [2,3]. RIBE has been observed in a range of cell types and tissues. Cell-cell communication in vivo has been termed the radiation-induced abscopal effect (RIAE), which results in changes in a tissue or organ outside the irradiation field but within the same organism [4]. Bystander/abscopal cells manifest a collection of biological...
consequences, including mutagenesis, chromosomal aberrations, micronucleation, neoplastic transformation, cell death, augmented proliferation and differentiation [2,5,6].

Over the past decades, various traits of RIAE have come to light. Patients were reported to experience regression of tumours distant from the irradiation site [7-10]. On the other hand, following radiotherapy, RIAE in out-of-field normal tissues can contribute to development of radiation-induced secondary malignancies [1,11] and an increased incidence of inflammatory diseases [12,13]. Paediatric patients are more prone to radiation-induced carcinogenesis [14], as their bodies are still growing, and dividing cells have been shown to be more vulnerable to somatic genetic damage due to both targeted and non-targeted effects of radiation [15,16]. Besides radiation effects, there are other factors that also contribute to the development of secondary malignancies, such as genetic susceptibility and intrinsic radiosensitivity [17,18]. Therefore, contributing factors should be studied and considered in treatment planning.

There are several mechanisms that have been proposed to regulate the RIBE. These involve the transmission of signalling molecules, which include cytokines, growth factors, free radicals, reactive oxygen and nitrogen species (ROS and RNS), either via gap junctions or secretion [19-21]. These signalling molecules interact with healthy “naïve” neighbouring cells and have the capacity to cause biochemical alterations. Non-targeted effects in vivo, where the initial DNA damage response (DDR) at the irradiation site activates systemic responses, involve the immune system and mediators, such as cytokines.

High dose-rate synchrotron radiation is an attractive tool commonly used to study non-targeted effects of radiation due to its precise geometry and low scatter from the parallel X-ray beam [22]. Additionally, synchrotron facilities afford the chance to perform microbeam radiation therapy (MRT). The MRT beam is comprised of an array of planar separate microbeams produced from a uniform parallel X-ray beam that is passed through a collimator. MRT is a pre-clinical modality that has shown great promise for treating cancer [23,24]. Recent reports have highlighted its ability to effectively ablate tumours while also sparing normal tissues [25]. The improved therapeutic benefit provided by MRT is thought to be associated with the fact that it delivers extremely high doses to a tumour while simultaneously preserving normal tissues due to the presence of the low dose “valley” region within the MRT dose profile [26,27]. The mechanism that supports normal tissues ability to tolerate these high doses is still poorly understood. However, various explanations have been proposed, such as rapid regeneration of vasculature [25], increased tumour cell migration [28], increased stem cell proliferation and stem cell survival in normal tissues [29], immune response alterations in tumour and normal tissues [30,31] and RIBE [32,33].

It is still not clear how irradiation settings affect out-of-field normal tissues. It has been reported that increased radiation field size and irradiation modality can increase the risk of these radiation-associated side effects [14]. However, in vitro experiments have demonstrated that the RIBE is dose-independent [34,35]. To address this question and to determine how radiation dose and irradiated volume impact response for out-of-field normal tissues, the systemic effects of synchrotron radiation in a mouse model have been examined [36].

2. Results

We used a combination of doses and beam configurations to irradiate mice on the right hind leg (Figure 1). After 1 and 4 days post-irradiation a selection of out-of-field normal tissues and blood samples were collected. Abscopal effects can be measured using a variety of key endpoints, including ROS [37,38], DNA damage [39-41], apoptosis [42], inflammation [43], immune response [44], cytokine concentrations [45,46], oxidative stress [47], senescence [48,49] and proliferation [50]. Our findings can be summarised as follows: in out-of-field normal tissues, all radiation settings induced 1) significant and prolonged oxidative DNA damage; 2) persistent double stranded breaks (DSBs) were induced by most irradiation settings although the effect was dose-dependent and tissue specific; 3) increasing trend of apoptotic cell death; 4) increase of oxidative and inflammatory in-situ markers; 5) decreased proliferation and increased occurrence of senescent cells; 6) innate and adaptive immune
effector responses. In addition, we found alterations in plasma levels of pro-inflammatory cytokines (Figure 1).

![Figure 1](image-url). A schematic diagram of the propagation of systemic effects *in vivo* following localised BB and MRT synchrotron radiation. At the directly irradiated site, cells that are damaged and stressed, and immune cells are activated, releasing signalling molecules to adjacent tissues. This results in modifications to cytokine levels that can either directly or via other factors stimulate macrophages, neutrophils and T-cells in distant tissues. The imbalance that occurs in the local immune microenvironment causes the development of free radicals that consequently results in persistent oxidative stress. This leads to persistent and significant increases of OCDLs across all tissues in the body. Proliferative tissues, such as duodenum, endured significant DSBs due to unrepaired OCDLs, which ultimately encourages apoptotic cell death. The out-of-field duodenum also underwent alterations in proliferation, inflammation and senescence.

2.1 Oxidatively-induced clustered DNA lesions (OCDLs).

The OCDLs were consistently and significantly elevated across all out-of-field tissues, regardless of distance from the irradiation site, in a dose- and volume-independent manner. Bystander and abscopal effects have been documented to occur at distances up to 1 cm from the irradiated site and beyond [51-53]. We observed increases in OCDL frequencies in colon and duodenum, which are 1.5 cm from the irradiated site. Remarkably, OCDLs also increased in a tongue, which is 7 cm from the irradiation site. The lack of decline in OCDLs at 4 days post-IR was attributed to repair-resistance of OCDLs and/or persistent oxidative stress [54,55].

2.2 DSBs.

We monitored DSBs using the γ-H2AX foci assay [56]. DSBs consistently increased for out-of-field duodenum after exposure to lower but not higher peak doses. We attributed this observation to differences in γ-H2AX kinetics in directly irradiated and naïve abscopal cells. In the former, the peak γ-H2AX response is quick; the majority of radiation-induced foci disappear within hours, while in the latter, the response is slow and prolonged [51]. To further complicate this puzzle, non-targeted
radiation-induced damage increases as the dose decreases; the effect is opposite in directly irradiated tissues [57,58].

2.3 Apoptosis.
Frequencies of apoptotic cells immunostained by anti-cleaved caspase 3 antibody were generally elevated in out-of-field duodenum.

2.4 Immune cells.
We established changes in frequency of macrophages/dendritic cells, neutrophils and T-lymphocytes in out-of-field duodenum, suggesting the involvement of both innate and adaptive immune responses. At the locally irradiated site (skin), macrophages/dendritic cells and neutrophils also increased, with the exception of T-lymphocytes.

2.5 Plasma cytokines.
A range of reports have provided compelling evidence that cytokines play a key role in non-targeted bystander signalling [45,46]. We selected a panel of 20 cytokines associated with inflammation, DNA damage response and immune response. Blood plasma was screened for expression levels of the selected cytokines; 7 of the 20 cytokines tested were consistently and significantly altered. These include: eotaxin, macrophage derived cytokine (MDC), interleukin-10 (IL-10), TIMP metallopeptidase inhibitor 1 (TIMP-1), vascular endothelial growth factor (VEGF) and transforming growth factor beta 1 and 2 (TGFβ-1 and TGFβ-2). Some of these changes were similar to those seen in our previous study that involved plasma cytokine modulations in patients treated with fractionated radiotherapy [21,59], while some were different. The discrepancies between both studies could be attributed to differences in systemic responses between mice and humans, and between single dose and fractionated radiation treatments. In any case, alterations in cytokine expression indicate that the radiation settings induced an imbalance in the environment.

2.6 Proliferation, senescence, and oxidative and inflammatory in situ markers.
In vivo, persistent oxidative stress often leads to loss of homeostasis and to pathogenesis, such as inflammation and cancer [60]. A prime candidate that is often associated with increased oxidative stress is ROS, as they are released by directly irradiated cells or activated macrophages [61,62]. In out-of-field duodenum of BB-irradiated mice, we found increases in 8-oxoguanine a marker for oxidative stress, increases in p65, which is associated with inflammation, decreases in Ki67-positive cells, a marker for proliferation, and decreases in senescence using the Sudan Black B assay [63,64]. All of these responses were consistently and significantly altered across all BB-irradiated groups and persisted for 4 days post-irradiation.

3. Conclusions
In conclusion, we found that both MRT and BB were capable of inducing persistent systemic oxidative stress, genotoxic events and immune response, generally in a dose-independent manner. These observed bystander effects were not largely influenced by beam modality, dose and size of irradiation field. The γ-H2AX response at the higher dose differed from all other endpoints, which we believe is due to the contribution of scattered radiation confirmed by our dosimetry studies [26,40]. Our report is in agreement with the idea that macrophages and cytokines are key players in propagating the systemic effects of ionising radiation. We have confirmed that innate and adaptive immune responses influence non-targeted effects and we now aim to establish which combination of immune system components drive its propagation. This is the subject of another study, which involves the use of mice with defects in their immune system treated with similar radiation conditions described in this study. Clinical studies will validate the significance of our findings for planning of MRT and ablative radiotherapy treatment.
4. Acknowledgements
This study was mainly supported by the Australian National Health and Medical Research Council (NHMRC) grant #10275598. We thank the Australian Synchrotron for providing facilities and continuous support.

5. References
[1] Prise K M et al 2009 Nat. Rev. Cancer 9 351-60
[2] Mothersill C et al 2004 Nat. Rev. Cancer 4 158-64
[3] Hamada N et al 2007 J. Radiat. Res. 48 87-95
[4] Mole R H 1953 Brit. J. Radiol. 26 234-41
[5] Azzam E et al 2004 Curr. Cancer Drug Tar. 4 53-64
[6] Hei T K 2006 Mol. Carcinog. 45 455-60
[7] Kaminski J M et al 2005 Cancer. Treat. Rev. 31 159-72
[8] Mancuso M et al 2012 Curr. Mol. Med. 12 613-24
[9] Shiraishi K et al 2008 Clin. Cancer Res. 14 1159-66
[10] Siva S et al 2015 Cancer Lett. 356 82-90
[11] Hall E J et al 2006 Int. J. Radiat. Oncol. 65 1-7
[12] Essen C F 1991 Clin. Exp. Metastas. 9 77-104
[13] Ishiyama H et al 2012 Clin. Genitourin. Canc. 10 196-8
[14] Kamran S C et al 2016 Cancer 122 1809-21
[15] Sprung C N et al 2005 J. Neuro-Oncol. 4 26-38
[16] Dickey J S et al 2012 Nucleic Acids Res. 40 10274-86
[17] Flint-Richter P et al 2007 Lancet Oncol. 8 403-10
[18] Zhou H et al 2005 Signaling Pathway 14641
[19] Shao C et al 2003 FASEB J. 17 1422-7
[20] Sprung C N et al 2015 Cancer Lett. 368 191-7
[21] Lobachevsky P et al 2015 Radiation Research 184 650-9
[22] Dilmian A et al 2002 J. Neuro-Oncol. 4 26-38
[23] Dilmian A et al 2003 Radiat. Res. 159 632-41
[24] Bouchet A et al 2010 Int. J. Radiat. Oncol. 78 1503-12
[25] Curtis H J 1967 Radiat. Res. 7 258-64
[26] Laissue J et al 2007 Dev. Med. Child Neuro. 49 577-81
[27] Crosbie J C et al 2010 Int. J. Radiat. Oncol. 77 886-94
[28] Zhong N et al 2003 Radiat. Res. 160 133-42
[29] Sprung C N et al 2012 Radiat. Res. 178 249-59
[30] Smilowitz H M et al 2006 Neuro-Oncology 78 135-43
[31] Mothersill C et al 2014 Dose Response 12 72-92
[32] Smith R W et al 2013 Int. J. Radiat. Biol. 89 118-27
[33] Schettino G et al 2005 Radiat. Res. 163 332-6
[34] Smith L B et al 2006 Radiat. Prot. Dosim. 122 256-9
[35] Ventura J et al 2017 Cancer Res. 77 6389-99
[36] Belyakov O et al 2002 Radiat. Prot. Dosim. 99 249-51
[37] Coates P J et al 2008 J. Pathol. 214 610-6
[38] Chou C H et al 2007 Clin. Cancer Res. 13 851-7
[39] Narayanan P et al 1999 Radiat. Res. 152 57-63
[40] Bartek J et al 2008 Nat. Cell Biol. 10 887-9
[41] Belyakov O V et al 2003 Br. J. Cancer 88 767-74
[51] Sedelnikova O A et al 2007 Cancer Res. 67 4295-302
[52] Belyakov O V et al 2005 P. Natl. Acad. Sci. USA 102 14203-8
[53] Koturbash I et al 2016 Mutat. Res. 787 43-53
[54] Georgakilas A G 2008 Mol Biosyst. 4 30-5
[55] Gollapalle E et al 2007 Radiat. Res. 167 207-16
[56] Bonner W M et al 2008 Nat. Rev. Cancer 8 957-67
[57] Seymour C B and Mothersill C 2000 Radiat. Res. 153 508-11
[58] Suchowerska N et al 2005 Phys. Med. Biol. 50 3041-51
[59] Siva S et al 2014 PloS one 9 e109560
[60] Sedelnikova O A et al 2010 Mutat. Res. 704 152-9
[61] Hussain S P et al 2003 Nat. Rev. Cancer 3 276-85
[62] Tartier L et al 2007 Cancer Res. 67 5872-9
[63] Georgakopoulou E A et al 2013 Aging 5 37-50
[64] Evangelou K et al 2016 The Specific Histochemical Stain for Lipofuscin: A Novel Method to Detect Senescent Cells. New York, NY: Humana Press 111.