AN *IN VITRO* INVESTIGATION OF THE IMPACT OF RADIATION INDUCED BYSTANDER EFFECT ON THE THERAPEUTIC IRRADIATION OF A PROSTATE CANCER CELL LINE.

Thesis by publication submitted for the degree of
Master of Science in Medical Physics
by

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Abstract

Introduction.

The aim of radiotherapy, in general, is to deliver a high enough radiation dose to tumour cells to control (and stop) their growth without causing severe complications to surrounding healthy tissues. As a result, it is very important to define a precise irradiation target for radiotherapy treatment. For many years only DNA has been seen as the main target for radiation to cause cellular death in living tissues. In the last decade the fundamental dogma of radiobiology, known as the 'target theory', has been reviewed. The extensive experimental evidence demonstrates that not only cell nucleus but also cellular cytoplasm, membrane, and even neighbouring cells, located outside the radiation field, should be viewed as possible targets for therapeutic ionising radiation.

Methodology.

The research described in this thesis aims to investigate the impact of the non-targeted effects of 6MV x-rays during the radiotherapy. This thesis intends to analyse the published mathematical models which predict occurrence and magnitude of radiation induced bystander effects (RIBEs), with experimental validation of one of these models. The methodology undertaken involved:

- Literature review and development of comprehensive understanding of general concepts of radiation induced bystander effects;
- Establishment of a suitable experimental methodology to investigate these phenomena, in particular radiation induced additional killing, in the application to radiotherapy to PC3 human prostate epithelial adenocarcinoma cell line, including:

    - evaluation of biological characteristics such as population doubling time and plating efficiency;
    - evaluation of radiobiological characteristics such as the dose which kills half of clonogenes (D_{50}), which will be used subsequently as the prescribed dose in the dose cold spot experiment; (in the experiment investigating cell survival in the under-dosed region)
    - determination of suitable biological end-points (such as apoptotic cell death, reduced proliferation rate, clonogenic cell death) following radiation treatment;
    - design of a dose-cold spot experiment to investigate RIBE in a reduced dose region (ie receiving ~80% of the prescribed dose) in freely communicating cells and non-communicating cells;
- Investigation of the extent of non-targeted effects on cell killing in a dose cold spot in human prostate PC3 cancer cell line;
- Analysis of RIBE related models;
- Validation of the published stochastic model that relates absorbed dose to the emission and processing of cell death signals by non-irradiated cells which included:
    - determination of magnitude of medium-borne signals (affecting non-targeted cells) dependence on the radiation doses received by donor cells
    - investigation of donor cell concentration impact on the emission of death signals predicted by the model.

All cell irradiations were performed at the Royal Adelaide Hospital, Radiation Oncology Department using a 6 MV x-ray beam produced by a Varian linear accelerator (Varian, Palo Alto, CA,USA). A clinically applied nominal dose rate of 3 Gy/min was used. Each radiation treatment

was performed at 100 cm from the beam focal spot with 20 x 20 cm^2 radiation field size. The culture dishes were placed on the top of 1.5 cm thick solid water build up sheets. To avoid irradiation through air gaps cells were treated posteriorly with gantry positioned at 180^o. Custom made wax phantoms (for different flask sizes) were used in conjunction with 5 cm thick solid water slab to cover the flask to ensure full scatter conditions. Machine radiation output was routinely checked with Daily QA 3™ device (Sun Nuclear, USA) before each radiation treatment.

The primary research objectives were investigated through a series of research papers.

Results.

The findings and results of the experiments designed and performed in the current work include:

I. Biological characteristics of PC3 cell line such as plating efficiency and population doubling time were found to be 0.60, 48 hours respectively.

II. The fraction of cells surviving the standard clinical daily dose of 2 Gy (SF2) typical of curative radiation protocols was found to be 0.586 (± 0.0279), while the dose that killed half of the clonogen population (D_{50}) was found to be 2.037Gy.

III. Radiosensitivity of PC3 cells differs widely among laboratories - the maximum difference found was 131.58%. This cell line appeared to be very sensitive to the methods used therefore it was important to evaluate D_{50} independently rather than relying on published data.

IV. Apoptotic assay revealed no significant dose dependant early cell deaths until 96 hours after radiation exposure. Following this time the first sizable colonies can be detected by the clonogenic survival assessment. Hence cellular damage in a dose cold spot was assessed by long term survival data which includes all types of radiation induced damages.

V. Cells exposed to a dose cold spot that are freely communicating versus non-communicating cells revealed significant decrease (16.2%) in cells survival presumably due to intercellular communication.

Validation of the stochastic model predicting emission and processing of cell death signals in non-irradiated cells revealed significant decreases in cell survival ($P<0.001$) exposed to irradiated cell condition media (ICCM) derived from donor cells of various concentrations and irradiated with different doses. Dependency of the toxicity of ICCM on the cellular concentration of donor cells was fond to be significant ($p<0.5$) as well.

Conclusion.

For the given cell line under existing growing and treatment conditions the cell survival in the dose cold spot region was significantly lower when under-irradiated cells were in contact with the cells receiving 100% of the prescribed dose compared to the cellular survival obtained from the under-dosed cells, by the same amount of radiation, which were treated separately. Presumably these variations were mainly due to intercellular communication.

Significant reduction in PC3 cell survival after receiving ICCM was observed. Data fitting revealed an exponential decrease in recipient cell survival with the dose received by the ICCM. However the current experiment was not able to identify the associated dose threshold for the reduction in survival from ICCM due to the saturation of the effect at the doses investigated. This can be attributed to either saturation in signal generation due to limited signal potency or saturation in recipient cell responses. It appeared that death signal emission may increase with increasing numbers of radiation hits to a certain target and with increasing number of targets able to emit death

signals. However, the effect saturates when it reaches a specific value in a number of hits or in an amount of critical targets.

The mechanisms behind radiation induced additional killing are not clear yet. Little is known about the types of DNA damage affecting bystander cells. The impact of RIBEs in application to novel radiotherapy treatment techniques, such as intensity modulated radiation therapy and tomotherapy, needs further investigation as they deliver highly conformal doses to tumours, but cover bigger volumes with the low doses where bystander responses are more pronounced.

Incorporation of RIBEs into the research that underpins clinical radiotherapy will result in a shift beyond simple mechanistic models currently used towards a more systems-based approach. It is a difficult task to design a coherent research strategy to investigate the clinical impact of bystander phenomena, given the complex protean nature of it. Any consideration of bystander effects will challenge clinicians' preconceptions concerning the effects of radiation on tumours and normal tissues and therefore disease management.

List of publication by candidate.

Published papers:

Sjostedt, S., and Bezak, E. (2010) "Non-targeted effects of ionising radiation and radiotherapy", Australas Phys Eng Sci Med 33, 219-231.

Sjostedt, S., Bezak, E. (2012) Experimental investigation of the cytotoxicity of medium-borne signals in human prostate cancer cell line", Acta Oncologica,. 04/2012. DOI:10.3109/0284186X.2012.670264

Sjostedt, S., Bezak, E. and Marcu, L. (2012) "Experimental Investigation of the Cell Survival in Dose Cold Spot in Communicating and Non-Communicating Cells", Submitted to Acta Oncologica.

Conference presentations:

- 'Review of the Radiation Induced Bystander Effect and its possible effects in radiotherapy' - Modelling of Tumour meeting 2 (Adelaide, SA, 2008);
- "Experimental investigation of the radiation induced bystander effect and its possible effect on cell survival in dose cold spots." - EPSM ABEC (Christchurch, New Zeeland, 2008);
- "Investigation of the human prostate PC3 cells' survival in a dose cold spot" – Modelling of Tumour meeting 3 (Adelaide, SA, 2010), student paper night (Adelaide, SA, 2010);
- "Experimental investigation of the cell survival in dose cold spots in communicating and non-communicating cells" – EPSM ABEC (Darwin, NT, 2011);
- "Experimental investigation of the cytotoxicity of medium-borne signals in human prostate cancer cell line" – ESTRO 31 (Barcelona, Spain, 2012).

Awards:

The best student poster presentation for “Experimental investigation of the radiation induced bystander effect on cell survival in dose cold spots.” - EPSM ABEC (Christchurch, New Zeeland, 2008).

Statement of Original Authorship.

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Statement of Contribution.

Cell line experiments reported in this thesis were designed and performed by myself. I carried out the background research, however the guidance and scientific advice were frequently provided by my Principal Supervisor A/Prof Eva Bezak.

I was trained and overseen by Dr Tony Cambareri and Dr Fares Al-Ejeh to handle cellular materials and to perform various biological assays.

Cell irradiations for all radiobiological experiments reported in this thesis were carried out by me and Eva Bezak. The beam-on-time parameters for each experimental setup were determined by myself and overlooked by my Principal Supervisor A/Prof Eva Bezak as well as the results scoring and corresponding statistical analysis.

Chapter 2.0 of this thesis contains a version of an article published in Australasian Physical & Engineering Sciences in Medicine as:

Sjostedt, S., and Bezak, E. (2010) "Non-targeted effects of ionising radiation and radiotherapy", Australas Phys Eng Sci Med 33, 219-231.

Sjostedt, S (candidate) performed literature review and wrote the manuscript.

I hereby certify that the statement of contribution is accurate

Signed: .....Date:.....

Bezak, E advised on data interpretation and edited the manuscript.

I hereby certify that the statement of contribution is accurate

Signed: .....Date:.....

Chapter 3.0 of this thesis contains a version of an article submitted to Acta Oncologica as:

Sjostedt, S., Bezak, E. and Marcu, L. (2012) "Experimental Investigation of the Cell Survival in Dose Cold Spot in Communicating and Non-Communicating Cells'.

Sjostedt, S (candidate) performed the experiments and data analysis, interpreted the results and wrote the manuscript.

Certification that the statement of contribution is accurate.

Signed: .....Date:.....

Bezak, E supervised the project, assisted with cells irradiation, advised on data interpretation, and edited the manuscript.

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

Signed: .....Date:.....

Marcu, L edited the manuscript.

Certification that the statement of contribution is accurate and permission is given for the inclusion of the paper in the thesis

Signed: .....Date:.....

Chapter 4.0 of this thesis contains a version of an article published in Acta Oncologica as:

Sjostedt, S., Bezak, E. (2012) Experimental investigation of the cytotoxicity of medium-borne signals in human prostate cancer cell line", Acta Oncologica,. 04/2012; DOI:10.3109/0284186X.2012.670264

Sjostedt,S performed the experiments and data analysis, interpreted the results and wrote the manuscript.

I hereby certify that the statement of contribution is accurate

Signed .....Date:.....

Bezak, E supervised the project, assisted with cells irradiation, advised on data interpretation, and edited the manuscript.

I hereby certify that the statement of contribution is accurate

Signed:…….Date:.....

Acknowledgement.

Completion of this Master project would not have been possible without support and assistance of numerous people. I would like to express my appreciation to my Principal Supervisor A/Prof Eva Bezak for her support, guidance and professional advice provided to me throughout my research project. I am very grateful for her assistance. It has been both a privilege and a pleasure to watch and learn from her.

I would also like to express my appreciation to staff of SA Pathology, Haematology Department for providing us with lab facilities, training me to handle cell line materials and to perform series of biological assays, especially to Dr Tony Cambareri and Mrs Sharon Paton.

Additionally I wish to acknowledge the generous support provided by Dr Fares Al-Ejeh, Senior Research Officer from Experimental Therapeutics Lab, Hanson Institute, for his scientific advice and assistance in performing the dose cold spot radiobiological experiment.

I also would like to express my gratitude to Prof Wayne Tilley and Dr Tina Bianco-Miotto from the Dame Roma Mitchell Cancer Research Laboratories, University of Adelaide and the Hanson Institute, for generously providing us with the cell line materials.

Furthermore, I would like to indicate my appreciation to all colleagues, family members and friends involved in editing and correcting my articles and this thesis, especially to my husband John Sjostedt, my friend Inna Rumokoy and my colleagues Dr Eva Bezak, Dr Justin Sheppard, Dr Loredana Marcu and Mr Joshua Morrees.

Finally my appreciation is extended to my colleagues at Medical Physics Department, Royal Adelaide Hospital, for their support throughout my research project.

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