IRISH RADIATION RESEARCH SOCIETY

2021 Scientific Meeting

16th – 17th September

Online Event

#IRRS2021

LET THE DISCUSSION BEGIN!

Thursday September 16th	
An All Ireland Radiation Research Platform	
18:00 – 18:05	**Opening Address** Dr. Laure Marignol, Trinity College Dublin, Chair, Irish Radiation Research Society Welcome to Irish Radiation Research Society Annual Scientific Meeting
18:05 - 18:30	Keynote lecture Professor Heiko Enderling, Smurfitt Quantitative personalised medicine oncology lab, Moffit Cancer Institute, USA Simulating patient-specific tumour immune ecosystem perturbation by radiotherapy
18:30 - 18:40	Professor William Gallagher, University College Dublin. The All Ireland Cancer Research Institute AICRI .
18:40 - 18:45	Dr. Laure Marignol, Trinity College Dublin, Chair, Irish Radiation Research Society An All Ireland Radiation Research Platform: Let's make it happen.
18:45 - 18:55	Ms Margaret Grayson, Patient Representative, Northern Ireland Cancer Research Consumer Forum, UK. An All Ireland Radiation Research Platform: Patient perspective.
18:55 – 19:05	Dr. Ciara Lyons, Consultant Radiation Oncologist, Cork, Ireland An All Ireland Radiation Research Platform: Clinician's perspective (IRE),
19:05 - 19:15	Professor Gerry Hanna, Consultant clinical Oncologist, Belfast, NI. An All Ireland Radiation Research Platform: Clinician's perspective (NI),
19:15 – 19:20	Dr. Gerard Walls, Dr. Orla Houlihan Northern Ireland An All Ireland Radiation Research Platform: A trainee perspective
19:20 – 19:30	General discussion.
19:30	***Close End of meeting***

Friday September 17th	
Radiation Research	
10:00 – 10:05 (5 minutes)	**Opening Address** Dr. Laure Marignol, Trinity College Dublin, Chair, Irish Radiation Research Society Welcome
10:05 – 10:15 (10 minutes)	Dr. Gerard Walls, Queen's University Belfast Irradiation of the heart base causes functional and transcriptomic changes in the conduction system in a preclinical model
10:15 – 10:25 (10 minutes)	Jason McGrath, Trinity College Dublin The influence of microRNA-31 on oxidative stress, DNA Damage, and radio-sensitivity in pancreatic adenocarcinoma
10:25 – 10:35 (10 minutes)	Croi Buckley, Trinity College Dublin Targeting energy metabolism to improve radioresponse in rectal cancer
10:35 – 10:40 (5 minutes)	Fiona O'Connell, Trinity College Dublin The influence of increasing radiation on immune-metabolic regulation in adipose tissue explants
10:40 – 10:45 (5 minutes)	John D. O'Connor, Queen's University Belfast A framework for testing transcriptomic cancer cell line radiosensitivity signatures
10:45 - 10:50 (5 minutes)	Jie Feng, Queen's University Belfast Radiosensitisation of Hypoxic HPV-negative Head and Neck Cancer Tumour Models Using the Combination of Metabolism Suppressing Drugs and Gold Nanoparticles
10:50 – 10:55 5 minutes)	Wen Wong, Queen's University Belfast Radiosensitisation of breast cancer cells by DNA damage response inhibitors: an in vitro study
10:55 - 11:10 (15 minutes)	***Break***

11:10 - 11:20 (10 minutes)	Dr. Michelle Leech, Trinity College Dublin Exploring hypoxia in prostate cancer with T2-weighted Magnetic Resonance Imaging radiomics and Pimonidazole scoring.
11:20 - 11:30 (10 minutes)	Aoife Cannon, Trinity College Dublin A novel role for the complement cascade in chemoradiation therapy resistant oesophageal adenocarcinoma
11:30 - 11:40 (10 minutes)	Rebecca O'Brien, Trinity College Dublin Investigating the role of the complement system in the radioresistance of rectal cancer
11:40 - 11:45 (5 minutes)	Jade Monaghan, Technological University Dublin Identification of Raman spectral biomarkers from lymphocytes and prognostic factors associated with radiation toxicity in high risk localised prostate cancer patients enrolled on the SPORT radiotherapy trial
11:45 - 11:50 (5 minutes)	Francisco Liberal, Queen's University Belfast Mitotic catastrophe is the main mechanism of action in ^{223}Ra treated cells
11:50 - 12:00 (5 minutes)	Dr. Victorial Dunne , Queen's University Belfast Targeting DNA-repair gene mutations in metastatic castration-resistant prostate cancer with radium-223.
12:00 - 12:05 (5 minutes)	Shannon Thompson , Queen's University Belfast Investigating how underlying model assumptions impact biological response
12:05 - 12:10 (5 minutes)	Sophie Bockhold, University College Dublin Exploring the Translational Challenge for Medical Radiation Applications and Protection Research
12:10 - 12:15 (5 minutes)	Jack Haberlin, Waterford Institute of Technology Radioactivity in the Irish Coastal Environment - Assessment of the temporal and geographical impacts of radiocaesium discharges from Sellafield to Irish coastal waters.

Poster-style presentations	
12:15-12:30	Letitia Mohamed-Smith, Queen's University Belfast Targeting cellular metabolism to enhance radiotherapy response in *KRAS* mutant non-small cell lung cancer.
	Tongchauan Wang, Queen's University Belfast Mannose Mediated Radiosensitisation in HPV-negative Head and Neck Squamous Cell Carcinoma
	Paul Cahoon, Queen's University Belfast Investigating the Role of the cGAS-STING Pathway in Mediating Radiation Induced Bystander Effects
	Dr. Claire Keary, Waterford Institute of Technology Environmental Radiation Monitoring: a collaboration between Waterford Institute of Technology and the Environmental Protection Agency
12:30	***Close***
IRRS Annual General Meeting ***(15 Minutes)***	

Irradiation of the heart base causes functional and transcriptomic changes in the conduction system in a preclinical model

GM Walls, Ghita M, Edgar KS, Gill EK, Cole AJ, Jain S, Overman LM, Queen RA, Lisgo SN, Watson CJ, Grieve DJ, Williams KJ, McWilliam A, van Herk M, Butterworth KT.

Purpose

Radiation cardiotoxicity affects one third of patients with intrathoracic cancers and is largely characterised by vascular, electrical and pump dysfunction months-years post-radical doses. Recent clinical studies suggest that the dose received by the superior heart correlates most with morbidity and mortality. This region contains several critical substructures, including the proximal coronary arteries and the sinoatrial and atrioventricular nodes. We investigated the longitudinal effects of radiation on the cardiac conduction system in a partial heart irradiation mouse model by characterising the transcriptional, histological and functional changes following base irradiation.

Material/Methods

8-week old C57BL/6 mice received 16Gy/1# to the cardiac base, middle or apex of the heart using image-guided radiotherapy. The base was defined as the caudal third of the heart volume, encompassing the atria and conduction system nodes. Electrocardiography was performed at baseline and 10-week intervals for one year. Mice aged 9 months similarly underwent 16Gy/1# base irradiation, and had electrocardiography and tissue collection at baseline and 30 weeks. Paraffin-embedded tissues were stained with haematoxylin and eosin and Masson's trichrome. Whole-organ spatial transcriptomics was used to characterise regional variations in gene expression.

Results

Time-dependent increases in the P wave duration and PR interval were observed in young base-irradiated mice, most apparent at 20 weeks post-irradiation. Changes resolved at 40 and 50 weeks. Similarly, P wave duration and PR interval were increased at 30 weeks in aged mice. QRS duration was not prolonged in any groups. Atrial myocardial fibrosis were not increased compared with controls at 30 weeks in the aged mice. ECG changes were not related to whole-heart DVH metrics. Spatial transcriptomic analysis (10X Visium) revealed distinct gene expression patters in the base versus the rest of the heart, and changes in marker genes for the conduction system in the irradiated volume.

Conclusion

The cardiac conduction system exhibits subacute and self-limiting dysfunction independent of fibrosis following irradiation of the sinoatrial and atrioventricular nodes. Differential radiation on expression of genes effects were identified linked to cardiac conduction and other cardiac cell populations, both in- and out-of-field. This research has potential implications for radiation oncologists treating both thoracic malignancies and refractory tachyarrhythmias.

The influence of microRNA-31 on oxidative stress, DNA Damage, and radio-sensitivity in pancreatic adenocarcinoma

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Background

Radioresistance remains to be a significant challenge in the management of patients with pancreatic adenocarcinoma (PDAC). MicroRNAs (miRs) are small non-coding RNA molecules which can inhibit translation. Recent studies have revealed that miRs play a key role in radioresistance by altering levels of reactive oxygen species (ROS) such as hydrogen peroxide (H2O2) by targeting key metabolic enzymes including glutathione peroxidase 8 (GPx8), which plays an important role in ROS detoxification. MiR-31 has previously been demonstrated to regulate radiosensitivity in other cancer types, but remains unstudied in PDAC. Here, we investigated the biological role and potential mechanisms of miR-31 in PDAC radioresistance.

Methods

PCMV-miR vector containing a miR-31 mimic was stably expressed into miR-31 deficient BxPC-3 cells. Additionally, the pmiRZip lentivector was used to suppress miR-31 in miR-31 abundant Panc-1 cells. Clonogenic assays were conducted to explore the role of miR-31 manipulation on radioresistance within our stable models by assessing cell survival. ROS-Glo and GSH/GSSG-Glo assays were performed according to the manufacturer's instructions. Expression of potential miR-31 targets were measured by western blot analysis.

Results/Discussion

In this study, we found that overexpressing miR-31 in PDAC cells displayed an increase in H2O2 levels and enhanced sensitivity to radiation treatment. Whereas, suppressing miR-31 reduced H2O2 levels and promoted resistance to radiation treatment. Additionally, modulating miR-31 altered levels of DNA damage induction and repair associated with the differences in ROS levels observed. GPx8 is an antioxidant enzyme which is responsible for H2O2 detoxification by using GSH as its substrate. Interestingly, online bioinformatics tools present the 3'UTR of GPx8 as a predictive target of miR-31. We show that modulating miR-31 alters GPx8 expression therefore regulating H2O2 detoxification, consequently promoting either a radioresistant or radiosensitive phenotype in PDAC cells.

Conclusion

Our study demonstrates for the first time that miR-31 modulation can regulate radiosensitivity in PDAC cell lines by altering levels oxidative stress, potentially by regulating GPx8 expression and therefore monitoring H2O2 detoxification. Overall, miR-31 presents as a promising therapeutic target for regulating radiosensitivity in PDAC cells.

TARGETING ENERGY METABOLISM TO IMPROVE RADIORESPONSE IN RECTAL CANCER

Buckley C[1], Heeran A[1], O'Brien R[1], Xiaofei Yin[2], Nugent T[1, 3], Donlon N[1, 4], Hafeez A[3], O'Ríordáin DS[3], Hannon RA[3], Meighan B[3], McCormick P[1], Dunne C[3], Larkin J[3], Brennan L[2], O'Sullivan J[1], and Lynam-Lennon N[1].

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Introduction: Resistance to neo-adjuvant chemoradiation therapy is a significant clinical problem in the management of rectal cancer. There is an urgent need to identify underlying mechanisms of treatment resistance and novel treatment strategies to improve treatment response. In this study, we investigated the role of altered metabolism in rectal adenocarcinoma, and the potential of Metformin, a clinically-approved anti-diabetic drug and metabolic modulator, as a novel radiosensitiser in rectal cancer.
Methods: The metabolome of pre-treatment rectal adenocarcinoma tissue biopsies, and rectal tissue from non-cancer patients was assessed by Liquid-Chromatography Mass-Spectrometry (LC-MS). Radio- and chemosensitivity of SW837 rectal and HCT116 colon adenocarcinoma cells were assessed using the gold-standard clonogenic assay. Mitochondrial function was assessed using fluorescent probes. Energy metabolism of cell lines and ex vivo rectal cancer biopsies was assessed using a Seahorse XFe analyser. Cell-cycle, apoptosis and DNA-damage were investigated by flow cytometry. Glutathione and mitochondrial function were assessed luminescent/fluorescent assays, respectively. Irradiations were performed using the X-Strahl RS225 cabinet-irradiator. Experiments in hypoxic conditions were performed using a Whitley H35-hypoxystation.
Results: Twenty-three intracellular metabolites significantly differed between rectal adenocarcinoma biopsies and non-cancer rectal tissue. High levels of oxidative phosphorylation were demonstrated in pre-treatment rectal cancer tissue biopsies. SW837 cells were significantly more resistant to radiation and chemotherapy (5-FU) and demonstrated altered cell-cycle distribution and enhanced radiation-induced DNA damage repair, compared to HCT116. SW837 cells demonstrated altered energy metabolism, with significantly reduced dependence on glycolysis, when compared to HCT116. Metformin treatment significantly inhibited oxidative phosphorylation, increased glycolysis and induced mitochondrial dysfunction in vitro. Importantly, Metformin significantly radiosensitised HCT116 and SW837 to a clinically-relevant dose of X-ray radiation. Metformin significantly affected hallmarks of radioresistance in vitro, including cell-cycle distribution, apoptosis and anti-oxidants.
Conclusion: We have demonstrated differences in the metabolome of rectal cancer, when compared to non-cancer rectal tissue ex vivo. We have identified metabolic alterations, cell-cycle and DNA damage repair as potential mechanisms underlying radioresistance in rectal cancer in vitro. We demonstrate that Metformin significantly alters tumour metabolism importantly, that Metformin treatment significantly radiosensitises HCT116 and SW837 cells, potentially via a compensatory activation of glycolysis and alteration to cell-cycle, apoptosis and anti-oxidants.

The influence of increasing radiation on immune-metabolic regulation in adipose tissue explants.

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Introduction

Oesophageal Adenocarcinoma (OAC) is the most strongly associated cancer with obesity. Approximately 75% of OAC patients are obese which results in chronic systemic low-grade inflammation, which is believed to drive carcinogenesis as well as influencing radiation treatment response. Changes in metabolic oxygen consumption rate (OCR) has been associated with radio-resistance in OAC patients, however the role fat plays and how it responds to radiation is not understood making it the focus of this study.

Methods

Following patient consent, fresh human ex vivo Visceral Adipose Tissue (VAT) was exposed to varying doses of radiation, 0 (mock irradiated), 2, 4, 6, 8 and 10 Gy. Plates were incubated in a humidified 37^{o}C, 5% CO2 incubator for 24 hours.

Agilent Seahorse Xfe24 was used to measure different metabolic readouts such as oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Results were normalised to weight. Irradiated Adipose Conditioned Media (iACM) generated above was then analysed via MSD 54 V-plex ELISA to assess changes in secretion of angiogenesis, chemokine, cytokine, inflammatory, TH17 or Vascular injury mediators. Levels of dendritic cell maturation following ACM exposure were analysed by flow cytometry to assess dendritic cell maturation.

Results

Fat explant energy metabolism showed significant increase for OCR and ECAR at 4 and 8 Gy radiation doses compared with mock irradiated doses. IFN-γ, IL-10, IL-12p70, IL-4, IL-6, IL-8, TNF-α, IP-10, MDC, GM-CSF, TSLP and VEGF-D all display increased secretion with increased radiation doses. IL-23, IL-27 and Flt-1 displayed decreased secretion with increasing radiation doses. DCs showed decreased expression of co-stimulatory molecules CD80 and CD86 following exposure to iACM treated with higher radiation doses.

Conclusion

We have demonstrated using fresh ex-vivo human fat samples from OAC patients, that higher dose radiation treatment can significantly change real time metabolic profiling which could be directly linked with the inflammatory profile in VAT in these patients. This work may provide some insight into the development of novel therapies that target cancer-obesity linked mechanisms in tumours.

A framework for testing transcriptomic cancer cell line radiosensitivity signatures

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Radiotherapy is an important component of modern cancer treatment with about half of all cancer patients undergoing radiation therapy at some point in their treatment course. Modification of radiation delivery and dose are not yet influenced by molecular characterisation of tumour cells. Transcriptomic signatures aiming to describe cancer cell sensitivity to ionising radiation have been developed using small scale microarray data coupled with *in vitro* assessment of cancer cell line radiosensitivity. Models have been both pan-cancer and tissue specific. Some signatures have reported utility in predicting clinical outcomes despite showing relatively low accuracy in *in vitro* validation. Independent *in vitro* validation is important given that previous works have shown that most of the transcriptome is associated with cancer outcomes due to cellular process (e.g., proliferation) related gene expression. This work aimed to investigate these aspects for a selection of published transcriptome-based radiosensitivity predictors. Cancer cell line datasets (NCI60 [n=59] and Cancer Cell Line Encyclopaedia (CCLE) [n=522]) with available *in vitro* radiosensitivity measurements (survival fraction at 2 Gy for NCI60 (SF2) and mean inactivation dose for CCLE (MID)) were utilised to test 7 published radiosensitivity signatures. Along with the published signatures, three model types allowing for benchmarking were fitted. Firstly, a model including genes from cellular processes was assessed. Secondly, control signatures (resampled from all genes on microarrays) of the same size were tested. Finally, a model using only the intercept value from the training set was fitted. The published models had equivalent accuracy to the median accuracy control signature (within 95% CI) for mean absolute error. The intercept only model outperformed some published models, particularly where the training sets were smaller in the NCI60. Low accuracy was observed both when using cellular processes or intercept only to predict either MID or SF2. Poor performance of signatures suggests a need for model improvement which may be aided by greater sample size, improved modelling methods, incorporation of multiomics and external validations. Further assessment of radiosensitivity signatures in clinical cohorts using suitable null hypotheses (e.g., comparison to random signatures) and adjustment for confounding is needed.

Radiosensitisation of Hypoxic HPV-negative Head and Neck Cancer Tumour Models Using the Combination of Metabolism Suppressing Drugs and Gold Nanoparticles

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Head and neck squamous-cell carcinoma (HNSCC) is a disease characterised by its aggressiveness and higher risk of recurrence. Radiotherapy (RT) has been widely applied to the primary and adjuvant treatment of HNSCC, with high tumour local regional control and cure rates, especially for localized disease. However, due to the anatomically sensitive locality of these tumours, adverse effects caused by radiotherapy can detrimentally impact patient quality-of-life. Gold nanoparticles (Au NPs) have been widely explored as pre-clinical physical sensitisers to ionising radiation. However, the efficacy of radiotherapy enhancement by Au NPs is compromised under hypoxic conditions. Herein, we present a novel strategy designed to enhance the radiotherapy response of HNSCC by combining Au NPs with atovaquone or papaverine, known inhibitors of oxidative phosphorylation (OXPHOS). Our results show that both atovaquone and papaverine decrease the oxygen consumption rate (OCR) by 80% and 70%, respectively. Moreover, both atovaquone and papaverine inhibit mitochondrial respiration, with significant reductions in basal respiration, maximal respiration, and ATP levels. Au NPs alone sensitised FaDu, CAL27 and CAL33 cells to radiation, achieving a dose enhancement factor (DEF) at 4Gy ranging between 1.18 - 1.59. Under hypoxia the magnitude of effect was abrogated with a maximal DEF of 1. 25.. Encouragingly, dose enhancement effects increased by combining the physical sensitisers with OXPHOS inhibitors, achieving a maximal DEF of 1.95 in FaDu cells. Similar effects were observed when Au NPs were combined with papaverine. Studies to elucidate the mechanism of radiosensitisation showed that the combined treatment resulted in a higher apoptosis induction. Taken together, the combined treatment of Au NPs and OXPHOS inhibitors potentially represents a promising approach for the treatment of locally advanced hypoxia HPV-negative HNSCC.

Key words: Tumor Hypoxia, OXPHOS Inhibitors, Oxygen Consumption Rate, Gold NPs, Radiosensitization.

RADIOSENSITISATION OF BREAST CANCER CELLS BY DNA DAMAGE RESPONSE INHIBITORS: AN IN VITRO STUDY

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Radiotherapy has an established role in cancer management, but its efficacy can be weakened by treatment resistance. An underlying mechanism of radioresistance is overactivation of the DNA damage response (DDR) in malignant cells. Key effector proteins of the DDR have been identified, some of which represent cancer therapeutic targets including the ataxia-telangiectasia mutated (ATM) and ataxia-telangiectasia and Rad3-related (ATR) kinases, as well as poly (ADP-ribose) polymerase (PARP). DDR inhibitors have been shown to increase tumour radiosensitivity, therefore their concurrent use in radiotherapy patients is increasingly being evaluated. However, results from clinical trials have been suboptimal, possibly due to an incomplete understanding regarding the influence of DDR inhibition on ionising radiation (IR) dose fractionation and sublethal damage repair.

This preclinical study aims to address these knowledge gaps by evaluating the radiosensitising ability of ATM inhibitor AZD0156, ATR inhibitor AZD6738 and PARP inhibitor olaparib, utilising MDA-MB-231 and MCF-7 human breast cancer cells for in vitro experiments. Clonogenic assays were performed to assess cell survival and sublethal damage repair after treatment with IR and DDR inhibitors. Immunofluorescence microscopy of 53BP1 was utilised to evaluate DNA double-strand break repair kinetics after various treatments. Modifications in cell cycle distribution were also investigated using flow cytometry.

Overall results indicate that AZD0156 is the strongest radiosensitiser, while AZD6738 and olaparib also demonstrated radiosensitising potential. All three drugs led to more unrepaired DNA double-strand breaks at 24 hours after IR, thus explaining their synergy with 24-hour fractionated IR. However, their effect on sublethal damage repair and cell cycle distribution still requires further clarification. Regardless, this study provides substantial preclinical data to establish the role of AZD0156, AZD6738 and olaparib as radiosensitising agents, therefore supporting further evaluation of their combination with radiotherapy in clinical trials.

Exploring hypoxia in prostate cancer with T2-weighted Magnetic Resonance Imaging radiomics and Pimonidazole scoring.

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Purpose/Objective

High levels of hypoxia are associated with a poorer prognosis in prostate cancer. Regions of a tumour with a high oxygen concentration are believed to be up to three times more amenable to radiation therapy than are hypoxic regions. Radiomics, which involves advanced image analysis and high throughput extraction of mineable precise quantitative imaging descriptors or features that serve as non-invasive prognostic or predictive biomarkers may be assist in the identification of hypoxia in prostate cancer.

Materials/Methods

The prostatic index lesion of 88 intermediate or high-risk prostate cancer patients' T2-weighted MRIs were analysed in this study. All patients received the hypoxia marker pimonidazole (PIMO) prior to radical prostatectomy at a dose of 500 mg per m2 body surface. PIMO hypoxic scores were assigned by an experienced pathologist was blinded to MRI. Radiomics feature extraction was performed using an evaluation version of RadiomiX (RadiomiX Research Toolbox version 20180831 (OncoRadiomics SA, Liège, Belgium)) for non-clinical use. 165 features were extracted based on the prostatic index lesion. The features extracted were morphological features, local intensity features, first-order statistics, intensity histogram features, fractal features and textural features.

Statistical Analysis

Hypoxia was modelled as PIMO <3 (not hypoxic) and PIMO score ≥ 3 (hypoxic). Highly correlated features ($\rho > 0.9$), features with near zero variance and linear combinations between features were first eliminated from further analysis. Multivariable logistic regression with Elastic Net regularization was utilised using 10 times repeated 10-fold cross-validation to select the optimal model hyperparameters, optimizing for area under the receiver operating characteristic curve (AUC). All features were standardized before modelling. The simplest candidate model (i.e., the model with the fewest non-zero coefficients) within one standard error of the best performing model was selected.

Results

The average (out of sample) performance based on the repeated cross validation using the ONESE model yielded an area under the receiver operating characteristic curve (AUC) of 0.6 ?0.2 for radiomics features only using the BEST model. The most important features were Shape-based.

Conclusion

This preliminary MRI-radiomics study of the index lesion in intermediate and high risk prostate cancer provides a basis for the hypothesis that radiomics may have a future role in the identification of hypoxia in prostate radiation therapy. Further study with a larger cohort and an independent validation cohort are warranted.

A novel role for the complement cascade in chemoradiation therapy resistant oesophageal adenocarcinoma

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Background: Resistance to chemoradiation therapy (CRT) in oesophageal adenocarcinoma (OAC) is a significant clinical challenge. Elucidating underlying mechanisms of treatment resistance is crucial to improving survival rates.

Materials and Methods: Tumour conditioned media was generated by culturing pre-treatment OAC biopsies for 24 h. Natural killer cells were isolated from whole blood by density centrifugation and using MojoSort™ Human NK Cell Isolation Kit. C3 and CFB mRNA expression was assessed by qPCR. Secreted C3 and C3a were measured by ELISA. An isogenic model of radioresistant OAC was established by chronically irradiating OE33 cells (OAC cell line). C3aR, C5aR, CD107a and IFN-γ expression were determined by flow cytometry. C3 was knocked-down using siRNA. Complement-mediated lysis was measured by the emission of fluorescent calcein AM from OE33 P and R cells after treatment with human serum.

Results: The central factor of the complement cascade, C3, is expressed in OAC tumours and is increased in pre-treatment OAC biopsies from patients (n=13) who have a subsequent poor response to neoadjuvant CRT ($p<0.05$). C3 is secreted from OAC tumours and correlates with complement factor B (CFB) mRNA expression, a component of the alternative complement activation pathway ($p<0.0001$). In vitro, radioresistant OE33 R cells had increased mRNA expression of C3 and CFB compared to radiosensitive OE33 P cells ($p<0.05$). Higher levels of C3 and C3a proteins were present in the microenvironment of OE33 R cells compared to OE33 P cells ($p<0.001$). Furthermore, the anaphylatoxin receptors C3aR and C5aR are highly expressed intracellularly by OE33 P and R cells. In co-culture experiments, OE33 R cells have a reduced capacity to activate anti-tumour natural killer cells compared to OE33 P cells (CD107a $p<0.01$; IFN-γ $p<0.05$). Transient knockdown of C3 in OE33 R cells resulted in increased CD107a expression on NK cells ($p<0.05$). In addition, OE33 R cells had a greater ability to prevent complement-mediated lysis, when compared to OE33 P cells ($p<0.01$).

Conclusion: This study highlights, for the first time, a novel role for the complement cascade in the resistance of OAC to CRT and highlights complement as a novel predictive marker of treatment response in OAC.

Investigating the role of the complement system in the radioresistance of rectal cancer

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Background

Poor pathological response to neoadjuvant chemoradiation therapy (neo-CRT) is a clinical problem in rectal cancer. There is a need to determine molecular factors influencing response to neo-CRT and identity novel predictive biomarkers. Evidence supports a role for the complement system in tumourigenesis and therapeutic response in cancer.

Methods

Radiosensitivity of colorectal cancer (CRC) cell lines (HCT116, SW837, HRA-19, SW1463) was assessed by clonogenic assay. Cells were irradiated using an Xstrahl RS225 X-ray irradiator at a dose rate of 1.74 Gy/min. Complement gene expression was assessed by qPCR. Protein expression and anaphylatoxin production was assessed by ELISA. Transient knockdown of C3 was achieved by reverse transfection using siRNA. Expression of complement regulatory proteins and receptors was determined by flow cytometry. Circulating C3a levels in pre-treatment rectal cancer patient sera (n=39) were assessed by ELISA.

Results

HRA-19 cells are significantly more radioresistant when compared to SW837 and HCT116 cells, whilst HCT116 cells are the most radiosensitive. Complement proteins (C3, C5) and anaphylatoxins (C3a, C5a) were produced by CRC cells, with significantly lower levels produced by HCT116 cells. Complement protein production positively correlated with surviving fraction at a clinically-relevant dose of 1.8Gy X-ray radiation. CRC cells expressed complement regulatory proteins (CD46, CD55, CD59), and complement receptors extracellularly (C5aR) and intracellularly (C3aR, C5aR). Knockdown of C3 in HRA-19 cells was associated with increased radiosensitivity at 2Gy of ionising radiation. C3a levels were elevated in pre-treatment sera from rectal cancer patients with a subsequent poor pathological response to neo-CRT, when compared to good responders (p=0.039).

Conclusion

Complement is produced by CRC cells, with increased gene expression and protein levels associated with radioresistance. CRC cells expressed complement receptors and regulatory proteins suggesting they can respond to complement signals and modulate complement activation, respectively. Transient C3 knockdown resulted in increased radiosensitivity suggesting a functional role for complement in the radioresponse in CRC. In rectal cancer patients, increased C3a levels in pre-treatment sera was associated with a subsequent poor response to neo-CRT, further supporting a role for complement in treatment response and highlighting the potential for complement as a biomarker predicting response to neo-CRT in rectal cancer.

Identification of Raman spectral biomarkers from lymphocytes and prognostic factors associated with radiation toxicity in high risk localised prostate cancer patients enrolled on the SPORT radiotherapy trial

Jade Monaghan[*1,2], Daniel Cullen[1,2], Dinesh Medipally[1,2], Kelly Redmond[3], Ciaran Fairmichael[3,4], Suneil Jain[3,4], Aidan Meade[1,2] and Fiona M. Lyng[1,2]

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High risk localised prostate cancer (PCa) accounts for 15% of patients diagnosed with PCa. Radiation is a standard treatment modality for prostate cancer patients and patients enrolled in this study received stereotactic ablative body radiotherapy (SABR). Two SABR treatment modalities were investigated and compared in this clinical trial: arm (PS): SABR to the prostate and to seminal vesicles alone and arm (PL): SABR to the prostate and to seminal vesicle alone with elective pelvic nodal irradiation (ENI). After treatment, PCa patients can experience toxicity in gastrointestinal and genitourinary tissues/cells and there is patient variability in response due to individual radiosensitivity. Optical spectroscopic methods such as Raman spectroscopy can provide a rapid, label-free and non-destructive measurement of the biochemical content of cells and biofluids. This study aims to elucidate the differences in the biochemical content of lymphocytes from high-risk localised prostate cancer patients through an intra and inter-radiotherapy treatment comparison to facilitate the prediction of intrinsic radiosensitivity.

PCa patients (n=30) were recruited as part of the SPORT High-Risk trial and blood samples were collected at baseline, post–hormone therapy, post-radiotherapy and 3 – month follow up. Clinical features such as baseline prostate-specific antigen (PSA), baseline citrulline and Gleason score were also recorded to explore their association with late radiation toxicity.

No correlation was observed between the clinical pairs and do not appear to be useful predictors of late toxicity in both treatment arms. Raman spectra were recorded from lymphocytes and the data was analysed using MATLAB software. Patient spectra were processed and classified by principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA). Statistically significant differences were observed in vibrational signatures between patients who developed toxicity in both intra and inter-radiotherapy treatment comparisons.

Overall, sensitivities and specificities achieved by the PLS-DA model were low with the exception of late toxicity prediction for treatment arm PS in the intra-radiotherapy treatment comparison. The results from this exploratory study suggest that Raman spectroscopy has some potential to predict radiosensitivity and classification rates may be improved by increasing the number of patients enrolled in future studies.

Mitotic catastrophe is the main mechanism of action in 223Ra treated cells

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Alpha-particle emitting radionuclides have been increasingly used in cancer treatment. However, 223Ra mechanism of action is unclear. There is a pressing need to model and quantify 223Ra effects in pre-clinical models so the next generation of trials can be optimally designed.

The present investigation was carried out to evaluate the radiobiological effect of 223Ra in 3 different prostate cell models (PC-3 and U-2OS metastatic lines, and normal RWPE) by assays of clonogenic survival and DNA damage.

Clonogenic cell survival curves were analyzed after irradiation with 225 kVp X-rays (dose rate 0.594 Gy/min), external α-particles (241 Americium) (dose rate 1.579 Gy/min) and 223Ra (dose rate 1.389 mGy/min). The results showed a superior efficacy of 223Ra in comparison with the external α source but with a cell type dependency. The Relative Biological Effectiveness (RBE) for 50% survival for RWPE is 6.07 and 7.97, for external α-particles and 223Ra respectively. For U-2OS is 6.36 and 8.9 and finally for PC-3 the values are 3.63 and 7.47.

The induction and repair of DNA damage by different radiation qualities was analyzed by immunofluorescence (53BP1). The level of induction and the shape of the kinetics curves are radiation- and cell-specific with the highest induction of foci observed after X-ray irradiation, the lowest after external α irradiation, and 223Ra being slightly higher than external α particles. In terms of repair, foci induced by external α source or 223Ra are repaired approximately 3.5 times slower than the X-ray induced breaks. Data fails to show significant differences between induction of damage and repair between external alpha irradiation and 223Ra exposures.

Interestingly, exposure to 223Ra severely affects the nuclear structure with a significant number of cells undergoing mitotic catastrophe, features not seem to the same extent with external beam irradiation. This Radium specific increase in mitotic catastrophe was a good correlation with the increased radium efficiency to induce cell death in the studied cell models.

In conclusion, our results suggest that response to Radium-223 is cell-specific and that better effectiveness does not solely depend on the DNA damage complexity.

Targeting DNA-repair gene mutations in metastatic castration-resistant prostate cancer with radium-223

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Metastatic castration resistant prostate cancer (mCRPC) remains an incurable disease in men with a 5-year survival rate of 30%, therefore, there is an urgent need for novel treatments that can improve patient prognosis and survival.

Radiotherapy (RT) is the standard-of-care treatment for localized and locally-advanced prostate cancer. RT activates the DNA damage response (DDR) which comprises a dynamic network of signaling pathways essential for genomic integrity and contributes to intrinsic radioresistance. Ataxia telangiectasia mutated (ATM) and Rad3-related (ATR) are critical proteins activated by DNA double strand breaks. Combining RT with DDR inhibitors has been shown to be a therapeutic strategy to enhance anti-tumour efficacy.

Radium-223 (223Ra) is a targeted, short range (<100 μm) densely ionizing, α-particle-emitting radionuclide which selectively binds to mineral hydroxyapatite at areas of increased metabolic activity such as bone metastases and has demonstrated efficacy in the treatment of mCRPC. Recently, germline and somatic aberrations in genes involved in homologous recombination (HR) have been reported in approximately 30% of mCRPC patients, of which BRCA2 and ATM are the most frequent aberrations. A retrospective study has demonstrated a greater clinical benefit from 223Ra for patients that harboured genomic HR mutations. In agreement, in this study we show an increased therapeutic efficacy of 223Ra in prostate cancer cell lines with loss of specific DNA-repair genes, which is associated with greater residual DNA damage, apoptosis and mitotic catastrophe in comparison to cells with functional DNA-repair genes. Moreover, for the first time, we examined the efficacy of radionuclides combined with DDR inhibitors in mCRPC. Clonogenic survival assays show that 223Ra in combination with DDR inhibitors has a greater radiosensitisation and cell killing effect in prostate cancer DDR-repair CRISPR knockout cell models compared to wildtype cells. These studies highlight the potential of exploiting DDR inhibitors as a mechanism of attenuating the therapeutic efficacy of 223Ra in patients with and without DNA-repair gene mutations.

Investigating how underlying model assumptions impact biological response

Shannon Thompson, Kevin Prise, Stephen McMahon

Mechanistic models of DNA damage and repair provide a useful tool to study the biological response of different radiation qualities. However, these models can differ in their underlying assumptions with the effect of such assumptions on the predicted biological response not yet fully investigated. This work aims to quantify the effect of different assumptions within radiation damage models to determine which assumptions significantly influence the DSB distribution, and therefore the biological outcome. The TOPAS-nBio and MEDRAS models were used to investigate the impact of assuming a radially symmetric energy deposition around an ion track compared to the complete track structure, and assuming DSBs rather than SSBs as the initial damage event. The irradiation of a nucleus with 1 Gy was simulated in each model for a range of ions and energies. To study the influence of radial symmetry, energy deposition events were converted to DSBs using random-energy dependent sampling and the DSB distribution was recorded. The initial damage event was then similarly investigated by converting energy deposition events into SSBs before clustering into DSBs.

The nuclear distribution of DSBs was not significantly influenced when comparing radial symmetry against the full track structure, indicating that this assumption would have minimal impact on the predicted biological response. Changing the initial lesion from DSBs to SSBs saw little difference in DSB distributions but had a much greater effect on strand break yields. DSB and SSB yields became strongly dependent on nuclear size when detailed nuclear geometries were not considered, requiring a sensitive nuclear region to be defined. Using SSBs as the initial damage event also introduced a strong LET dependence, increasing DSB yield with LET, contrary to the constant yield found when using DSBs as the initial lesion. This LET dependence was comparable across different methods of defining SSBs, indicating these methods do not significantly influence DSB yield in a way which cannot be modified by appropriate adjustment of model parameters. This work provides an initial step to determine which model assumptions significantly impact different biological endpoints, providing a foundation to help identify key data required to benchmark and validate DNA damage models.

Exploring the Translational Challenge for Medical Radiation Applications and Protection Research

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Background

Task 5.1 of the EURAMED Rocc-n-Roll project aims to establish a set of consensus statements highlighting key challenges to clinical translation of medical radiation applications and related radiation protection research.

Methodology

A Delphi methodology has been employed to gain consensus. In the first Delphi round a multidisciplinary panel of 20 individuals was tasked with generating a wide range of statements regarding radiation-specific barriers to translation across four broad categories: Basic Research, Commercial Development, Clinical Implementation, Education and Training. The second Delphi round called upon a broader panel of 130 individuals to rate the extent to which they agreed (or disagreed) with each generated statement as a key translational challenge for radiation protection research. SurveyMonkey® was used to capture evaluations via 6-point Likert Scale (1 = Strongly Disagree, 2 = Disagree, 3 = Somewhat Disagree, 4 = Somewhat Agree, 5 = Agree, 6 = Strongly Agree). Consensus was defined as median ≥4 with ≥60% of responses in the upper tertile of the scale (i.e., Agree / Strongly Agree).

Results

Consensus was reached for 61 statements following two Delphi rounds. The statements with the strongest overall agreement were:

- "Robust and efficient database structures that facilitate research across different repositories / platforms through secure data storage and information exchange are needed" (88.60% (n = 101) Agree or Strongly Agree).
- "QA is a big challenge for AI based applications, especially with respect to meaningful testing and understanding / evaluating limitations" (84.26% (n = 91) Agree or Strongly Agree).
- "There is a need for multidisciplinary approaches to education and training that incorporate a team of educators with radiation protection expertise from a range of professions/disciplines" (83.93% (n = 94) Agree or Strongly Agree).

A third Delphi round is currently ongoing, and the final set of core translational challenges disseminated to inform the development of an innovation transfer roadmap.

Conclusion

The final consensus document, arising from task 5.1 of the EURAMED Rocc-n-Roll project, will: identify the key challenges to clinical translation of radiation protection research; facilitate the development of a framework to address these challenges; and inform the implementation of future research and development work.

Radioactivity in the Irish Coastal Environment - Assessment of the temporal and geographical impacts of radiocaesium discharges from Sellafield to Irish coastal waters

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Since the mid-1980s, the Irish statutory authority charged with the responsibility to monitor radioactivity levels in the environment have conducted a marine monitoring programme to assess the levels of artificial radioactivity at a range of locations throughout Ireland. The results of this monitoring, together with data from numerous research studies, have enabled the statutory authorities to assess the impact of artificial radionuclide sources to the Irish coastal environment, such as the authorised discharges of low-level radioactive waste into the Irish Sea from the Sellafield reprocessing complex in Cumbria (UK). Although discharges peaked in the mid-1970s, enhanced concentrations of some nuclides, such as 137Cs, persist in the environment as a result of more recent (admittedly small) discharges and the contribution of remobilised radionuclides from historically contaminated sediments.

Although a considerable body of data has been gathered on the distribution and behaviour of artificial radionuclides in the Irish coastal environment, comparatively few data exist on the distribution and behaviour of natural radionuclides in Irish coastal waters. To remedy this, an EPA-funded project, titled Radioactivity in the Irish Coastal Environment (RICE) was recently initiated which aims to revisit several sites from previous surveys to provide an update on artificial radioactivity levels, which will supplement existing data, and to widen the scope of previous surveys to include the measurement of natural radioactivity levels.

In this presentation, 137Cs activity concentrations in samples of the brown seaweed Ascophyllum nodosum collected at locations along the Irish coast during June and July 2021 will be reported and the results compared with those of previous seaweed surveys conducted in the early- to late 1980s. As in previous surveys, our data shows that, conforming closely to known current patterns in the Irish Sea and surrounding waters, 137Cs concentrations are highest along the north-eastern coast, with concentrations rapidly diminishing south of Dublin along the south-eastern coast, and with the smallest concentrations being detected in locations along the west coast. In absolute terms, however, present activity concentrations are approximately two orders of magnitude lower than those prevailing in the early 1980s, confirming the continual decrease in 137Cs concentrations in sea water and marine biota in Irish coastal waters.

Targeting cellular metabolism to enhance radiotherapy response in KRAS mutant non-small cell lung cancer.

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KRAS is a key driver oncogene in non-small cell lung cancer (NSCLC), playing a role in various pathways of growth, proliferation, and survival. These KRAS gain-of-function mutations are associated with a more aggressive phenotype and poorer prognosis, contributing to a low five-year survival rate of approximately 20% for NSCLC. As tumours accumulate KRAS mutations, cellular glucose metabolism becomes reprogrammed which has significant effects on treatment responses. These metabolic vulnerabilities can potentially be exploited in the treatment of KRAS mutant NSCLC's. Studies have shown that hexose sugars can inhibit tumour growth and enhance chemotherapy response by modulating glucose metabolism. However, the impact of altered glucose metabolism on radiotherapy response has not yet been demonstrated.

This study aims to characterise the impact of selectively altering hexose sugar metabolism on the radiotherapy response in KRAS mutant NSCLC. It is hypothesised that the metabolic shift seen with early and late stage KRAS tumours can be used to mediate radiosensitivity and that interference with the established dependency of tumour cells on glucose, through the addition of other hexose sugars could be a potential therapeutic strategy for selectively sensitising NSCLC tumour cells to radiotherapy. Our initial findings show that treatment with x-rays and mannose shows a significant but varied decrease in survival fraction across a panel of both KRAS heterozygous and homozygous mutant NSCLC cell lines. This preliminary data suggests that KRAS may have a functional role in radiotherapy response and that altering glucose metabolism with mannose can enhance the radiosensitivity response in this tumour type. However, the exact mechanism by which mannose exerts its effect is unknown and further mechanistic studies are required to validate these findings and develop optimised metabolic targeting strategies**.**

Mannose Mediated Radiosensitisation in HPV-negative Head and Neck Squamous Cell Carcinoma

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Mannose is a monosaccharide which has been widely used to treat the congenital disorder of glycosylation-Ib. Recently, mannose, a C2-site stereoisomer of glucose, has demonstrated significant potential with respect to inhibiting tumor growth when combined with several major chemotherapeutic drugs[1]. In this study, we are investigating if mannose could enhance the biological effectiveness of ionizing radiation against various HPV-negative head and neck squamous cell carcinoma (HNSCC) models. Growth curve studies revealed that mannose significantly decreased the growth potential of all HPV-negative cell lines tested, exhibiting a strong dose dependency effect. Furthermore, prolonged exposure to mannose (20mM) sensitized FaDu (SER =1.28) and CAL27 (SER=1.29) cells to the effect of radiation treatment. However, scheduling of mannose treatment appeared to significantly impact the response, with no combined effect observed following short term mannose treatment pre and post IR administration. Preliminary 53BP1 immunofluorescence studies indicated that prolonged mannose exposure impairs the ability of tumor cells to adequately resolve radiation induced DNA double strand break. This resulted in a significant ($p=0.007$) increase in unresolved DNA DSB by approximately 68% over controls irradiated in the absence of mannose. We believe this may be linked to mannose-6-phosphate, a mannose intermediate metabolite, which has the ability to inhibit glycolysis, the tricarboxylic acid cycle, the pentose phosphate pathway and glycan synthesis[1]. Phosphomannose isomerase (PMI) plays an important role in converting mannose-6-phosphate to fructose-6-phosphate, implying that the cell's sensitivity to mannose depends on the level of PMI. Subsequent follow-on studies will be required to confirm this.

Key words: Mannose, HNSCC, DNA Damage Repair, Radiosensitization;

Reference:

1. Gonzalez, P. S. et al. Mannose impairs tumour growth and enhances chemotherapy. Nature 563, 719–723 (2018).

Investigating the Role of the cGAS-STING Pathway in Mediating Radiation Induced Bystander Effects

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Introduction

Radiotherapy is typically understood in terms of the direct effects of radiation on cells. However, there is an increasing focus on non-targeted effects (NTEs) of radiation, such as Radiation-Induced Bystander Effects (RIBEs) and the abscopal effect. RIBEs are the phenomenon of unirradiated cells exhibiting a response to irradiated neighbours, while abscopal effects relate to radiation's ability to provoke a response in vivo in unirradiated sites distant from the irradiated tumour, and are believed to be immune-driven. However, despite recent interest, the mechanisms governing NTEs remains elusive.

Cytoplasmic dsDNA is known to play a role in activation of the cGAS-STING pathway, a key driver of immune responses, and has also recently been implicated as a mediator of RIBEs. This research aimed to investigate if these two seemingly distinct processes share a common underlying pathway.

Methods

Clonogenic survival and co-culture assays were used to investigate cell survival in and out-of-field under different irradiation conditions. DNase I was added to MyC-CaP mouse prostate cancer cells, MDA231 breast cancer cells and MCF10A normal epithelial cells at various timepoints post irradiation. The involvement of the cGAS-STING pathway was further investigated by repeating these experiments using cell lines where either the cGAS gene or the STING gene were knocked out.

Results

Removing the damage signal (by degrading dsDNA) and inhibiting the sensing of this signal (by knocking out cGAS or STING) effectively abrogated the out-of-field cell killing associated with the RIBE. Also, co-culture survival assays showed that the cGAS-STING pathway of the bystander cells must remain intact for a RIBE to be observed.

Conclusion

Together, these results demonstrate how the cGAS-STING pathway, most frequently associated with abscopal immune responses, also plays a key role in mediating in vitro RIBEs. Additionally, cytosolic dsDNA, as the primary activator of this pathway, appears to be an important factor in the transmission of RIBEs between cells. In vitro RIBEs may be the manifestation of inflammation-driven genotoxic stress caused by downstream products of the cGAS-STING pathway.

Environmental Radiation Monitoring: a collaboration between Waterford Institute of Technology and the Environmental Protection Agency

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**Presenter*

Waterford Institute of Technology (WIT) and the Environmental Protection Agency (EPA) have developed a strategic partnership over a number of years in the area of environmental radiation monitoring. This partnership evolved through initial collaborations on undergraduate projects, the expansion of the laboratory facilities at WIT and the establishment of the Environmental Radiation Research Group (ERRG) at WIT. This has resulted in the commissioning of a second gamma ray spectrometry system and the installation of a high volume aerosol sampler unit at WIT, and ongoing collaboration on research projects between the two organisations including the EPA-funded Radioactivity in the Irish Coastal Environment (RICE) project.

A Service Level Agreement (SLA) has recently been agreed between the two organisations to provide a framework for enhanced cooperation in areas of mutual interest in environmental radiation monitoring. The Department of the Environment, Climate and Communications is responsible for Ireland's *National Plan for Nuclear and Radiological Emergency Exposures*; the EPA has been assigned a major role in the national response under the plan, with specific responsibilities assigned to its Radiation Monitoring Laboratory. A specific role has been delegated to WIT under the EPA's national radioactivity monitoring programme, namely to provide additional laboratory measurement capability to the EPA in the event of a nuclear or radiological emergency.

An overview of the evolution and development of this partnership and results from some past and current projects will be presented. The benefits to both organisations will be highlighted and plans for future work in the areas of environmental radiation monitoring and emergency preparedness will be outlined.