Purpose or Objective: Real time MR-guided radiotherapy is an emerging technology. The effect of magnetic field exposure on radiosensitivity is unknown. This study aimed to determine the effect of magnetic field exposure on the repair of radiation-induced DNA double-strand breaks in human prostate cancer cells.

Material and Methods: Human PC-3 prostate cancer cells and benign prostatic hypertrophy (BPH) cells were cultured and plated into 96-well dishes and irradiated with 2 Gy of 6 MV photons on a linear accelerator. Each cell line was exposed to either 2 Gy of ionizing radiation alone (IR) or 15 minutes of 0.2 T magnetic field concurrently with 2 Gy IR (IR + B). Cells were fixed at 15 minutes or 24 hours following IR and immunostained with fluorescent-labelled antibody to γH2AX, a marker of DNA double-strand breaks. For each experimental scenario, the number of γH2AX foci per cell were determined using a Molecular Devices MetaXpress High Content Imaging Platform, for sample sizes between 3370 and 8402 cells. To classify response, radiation-induced damage was associated with cells having more than five foci.

Results: Magnetic field exposure resulted in a significantly higher percentage of PC-3 cells with five or fewer γH2AX foci at 24 hours following IR (42 vs 37 percent, $p < 0.01$) but had no significant effect on BPH cells (89 vs 88 percent, $p = 0.26$). In both cell lines, magnetic field exposure significantly reduced the percentage of cells with five or fewer γH2AX foci 15 minutes following IR ($p < 0.01$) (Table 1).

Table 1. Percentage of BPH and PC-3 cells with≤ 5 γH2AX foci at 15 minutes and at 24 hours after exposure to 2 Gy of ionizing radiation alone (IR) vs 2 Gy of ionizing radiation with 15 minutes of concurrent 0.2 T magnetic field exposure (IR + B).

Time of cell fixation	BPH			PC-3		
	IR	IR + B	P-value	IR	IR + B	P-value
	Percentage of cells with ≤ 5 γH2AX foci			Percentage of cells with ≤ 5 γH2AX foci		
15 min	40	28	<0.01	17	14	<0.01
24 h	88	89	0.26	37	42	<0.01

Conclusion: The preliminary results suggest that the presence of a magnetic field during irradiation reduces DNA damage at 24 hours post-irradiation for PC-3 human prostate cancer cells. Conversely, magnetic field exposure increased the DNA damage present 15 minutes following IR in both cell lines, suggesting a different mechanism at play, such as altered free radical flux or differences in the kinetics of the initiation of the DNA damage response. Cell viability assays, gene expression profiling and testing of other cell lines will yield important insights into the implications for real time MR-guided radiotherapy.

EP-2069

CDC73 deficiency: a syndrome with multiple tumours is predicted to show excessive radiosensitivity

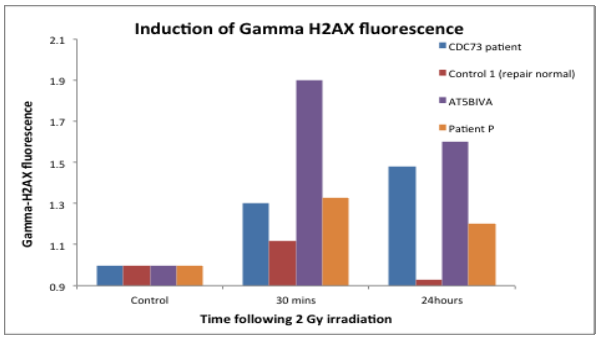
R. Lewis[1,2,3], E.C. Bourton[3], C.N. Parris[2,3], P.N. Plowman[1,2]
[1]*St Bartholomew's Hospital, Radiotherapy, London, United Kingdom*
[2]*Queen Mary's University, Barts Cancer Institute, London, United Kingdom*
[3]*Brunel University, Biosciences, Uxbridge- Middlesex, United Kingdom*

Purpose or Objective: It has previously been demonstrated that prolonged expression of the γ-H2AX DNA repair biomarker in irradiated peripheral blood lymphocytes correlated with excess toxicity from radiotherapy treatment in patients. γ-H2AX fluorescence in cells has been established asan indicator of double strand breaks, and a marker for DNA damage and repair of cells after irradiation. This case study illustrates that the peripheral blood lymphocytes of a patient with CDC73 deficiency retained γ-H2AX fluorescence over 24 hours to a greater degree than a patient with normal DNA repair.

CDC73 deficiency is an autosomal dominant inherited syndrome. The gene on chromosome 1q31 encodes a tumour suppressor that is known to be involved in transcriptional and post-transcriptional control pathways. The protein is a component of the PAF protein complex, which associates with the RNA polymerase II subunit POLR2A and with a histone methyltransferase complex, and is involved in regulation of transcription coupled nucleotide excision repair.
A patient with CDC73 mutation with a typical history of primary hyperparathyroidism, an ossifying fibroma of the jaw, renal cysts and a renal cell carcinoma developed a carotid body paraganglioma which was to be treated with stereotactic radiotherapy. There was concern that the syndrome (associated with multiple tumours) would lead to unusual radiation sensitivity following standard radiotherapy prescriptions, and this study aimed to establish if this would be the case.

Material and Methods: Peripheral blood lymphocytes (PBLs) from the patient were irradiated with 2Gy and fixed at 30 minutes and 24 hours, stained for γ-H2AX and compared with PBLs from a normal and radiosensitive patient (patient P - thyroid cancer with excessive toxicity to radiotherapy). They were also compared with known DNA repair defective immortalised fibroblasts from AT5BIVA (patient with classical ataxia telangiectasia). The cells were analysed on an Imagestream flow-cytometer.

Results:



Conclusion: It may be confidently predicted that this patient with CDC73 deficiency would demonstrate more vigorous radiation reactions in normal tissues for any standard dose of radiotherapy, due to a possible defect in DNA repair and this should be considered when planning his Cyberknife treatment for the carotid body paraganglioma. The exact mechanism for this will need to be considered along with current knowledge of the role of CDC73.

EP-2070

Cell cycle analysis of γ-H2AX in irradiated normal or DNA-defective cells with image flow cytometry

R. Lewis[1,2,3], P.N. Plowman[1,2], C.N. Parris[2,3]
[1]*St Bartholomews Hospital, Radiotherapy, London, United Kingdom*
[2]*Queen Mary's University London, Barts Cancer Institute, London, United Kingdom*
[3]*Brunel University, Biosciences, Uxbridge- Middlesex, United Kingdom*

Purpose or Objective: The quantitation of nuclear γ-H2AX foci in cells has been established as an indicator of double strand breaks, and therefore a marker for DNA damage and repair of cells after irradiation. The new generation image flow cytometer by Amnis Imagestream Mark II enables the rapid and simultaneous processing of images on multiple channels of large numbers of cells. It also has a unique feature or "wizard" which allows the identification of cell cycle distribution based on the fluorescence intensity of nuclear staining, in this case using the far red fluorochrome Draq5. This study aims to use this facility to establish whether there are different numbers of γ-H2AX foci in cells depending on the phase of the cell cycle. This is a novel