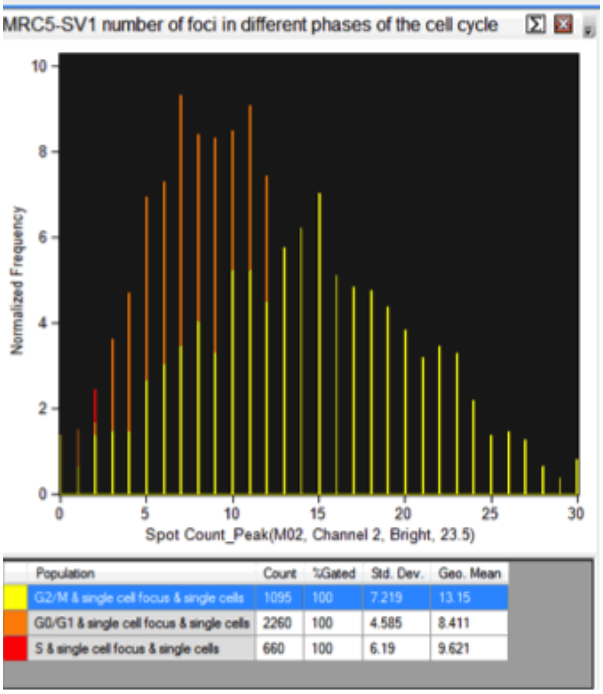
study and the outcome will inform future studies using γ-H2AX staining.

Material and Methods:
Fibroblast Cell Lines (SV40 immortalised)
– MRC5-SV1 - Repair normal.
– AT5BIVA – Classical ataxia telangiectasia.
Irradiation Cells
Irradiated with 2 Gy gamma radiation; harvested and fixed in 50:50 V:V methanol acetone. Time points: Un-irradiated, 30 min, 3, 5 and 24 hrs post irradiation.
Immunocytochemistry
Primary antibody: Anti-phospho-histone H2AX (Ser139), mouse monoclonal antibody clone JBW301 (1/10,000, Millipore).
Secondary antibody: Rabbit anti-mouse AlexaFluor488 (1/1000, Invitrogen).
DNA counterstained with Draq 5 (Biostatus Ltd.)
Imaging flow cytometry
Images of 5-10,000 cells captured

Results:



Statistical Analysis • 30 minute time point, comparing mean foci count for G0/G1, S and G2/M with one-way ANOVA test: – MRC5-SV1 (repair normal); $F(4,4010)=163.5$, $p < 0.001$ AT5BIVA (DNA repair defective); $F(2,2919)=421.3$, $p < 0.001$

Conclusion: We have identified cells in different phases of the cell cycle by analysing intensity of the Draq 5 nuclear stain and negating the need for extra staining. These data have shown a statistically significant difference between foci numbers in different phases of the cell cycle at one time point for a normal cell line and a DNA repair deficient cell line. Further work will look at differences in the cell cycle distribution between the two cell lines

Electronic Poster: Radiobiology track: Radiobiology of protons and heavy ions

EP-2071
Mitophagy and Apoptosis: mitochondrial responses to carbon ion radiation in tumor cells
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Purpose or Objective: Although mitochondria are known to play an important role in radiation-induced cellular damage response, the mechanisms by which tumor cells respond to the mitochondrial damage induced by high linear energy transfer (LET) radiation are largely unknown.

Material and Methods: Human cervical cancer cell line HeLa and human breast cancer cell lines MCF-7 and MDA-MB-231 were irradiated with high linear energy transfer (LET) carbon ions at low and high doses. Mitochondrial functions, dynamics, mitophagy, intrinsic apoptosis and total apoptosis, and survival fraction were investigated after irradiation.

Results: Compared with unirradiated cells, carbon ion irradiation resulted in the loss of mitochondrial membrane potential and fragmentation, suggesting mitochondrial damage was induced. Mitophagy and intrinsic apoptosis of tumor cells were the major responses to the carbon ion radiation induced mitochondrial damage. After exposure to low doses of carbon ions, cells initiated mitophagy to keep viability while tending to death via apoptosis at high doses.

Conclusion: Tumor cells through mitophagy and apoptosis respond to the mitochondrial damage caused by high-LET radiation according to the radiation dose. A threshold model depicting the fate of irradiated cells could provide a mechanistic explanation for differential mitochondrial damage response to high-LET radiation at low and high doses. Our data shed new light on understanding the mechanisms underlying high-LET radiation induced cell death.

EP-2072
Spatiotemporal dynamics of DNA damage in cells exposed to mixed beams of ionising radiation
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Purpose or Objective: A particular problem of modern external beam radiotherapy like IMRT and proton therapy is exposure of patients to scattered neutrons with a relative biological effectiveness (RBE) higher than X-rays. The interesting question is if there is an additive or synergistic effect of high and low linear energy transfer (LET) radiations when given together. If they act additively, then the risk of cancer can be deduced from the results of exposure to the single agents. Otherwise, RBE values must be generated for the mixed exposure scenarios or corrected to account for the synergism.

Material and Methods: The goal of this study was to analyse the kinetics of formation and repair of ionising radiation-induced foci (IRIF) in cells exposed to alpha particles, X-rays and a mixed beam of both radiations. To this end human cells were transfected with plasmids coding for the DNA repair the protein 53BP1 that are tagged with the green fluorescent protein (GFP). Cells were exposed to mixed beams in a dedicated exposure facility built at Stockholm University (SU). The facility is composed of a 50 MBq Am-241 alpha source and an YXLON 200 X-rays source. The alpha source is mounted on an inversed plate in a custom-designed irradiator which is kept inside a 37°C cell incubator.

Results: Spatiotemporal dynamics of 53BP1 foci formation and repair were recorded by time-lapse photography and image analysis. The distributions of cell frequencies with the specific size of foci and the size of foci itself were analysed. Moreover, Monte Carlo simulations (the PARTRAC code) were used not only for calculating radiation hits, but also for the biological damage in the DNA in terms of single and double strand breaks.

Conclusion: Exposure to a mixed beam induces complex DNA damage above the level expected from the additive action of