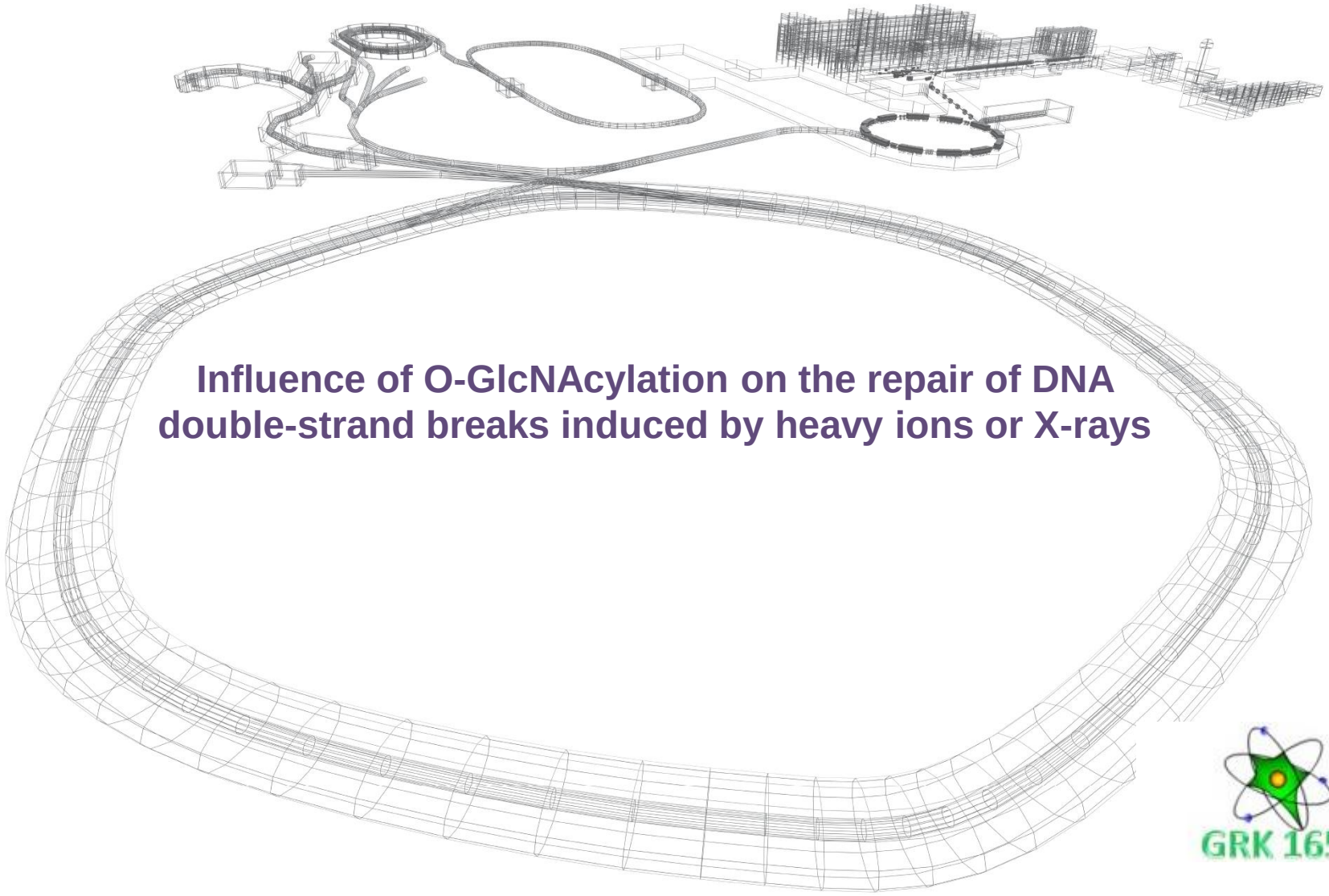
TECHNISCHE UNIVERSITÄT DARMSTADT

GSI

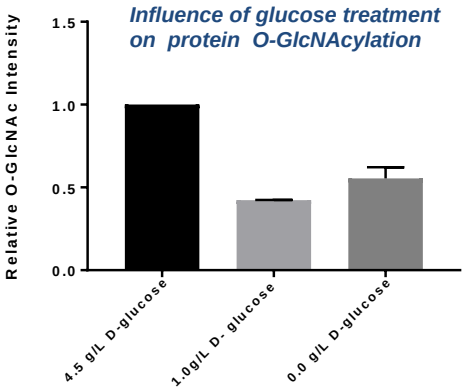


Influence of O-GlcNAcylation on the repair of DNA double-strand breaks induced by heavy ions or X-rays

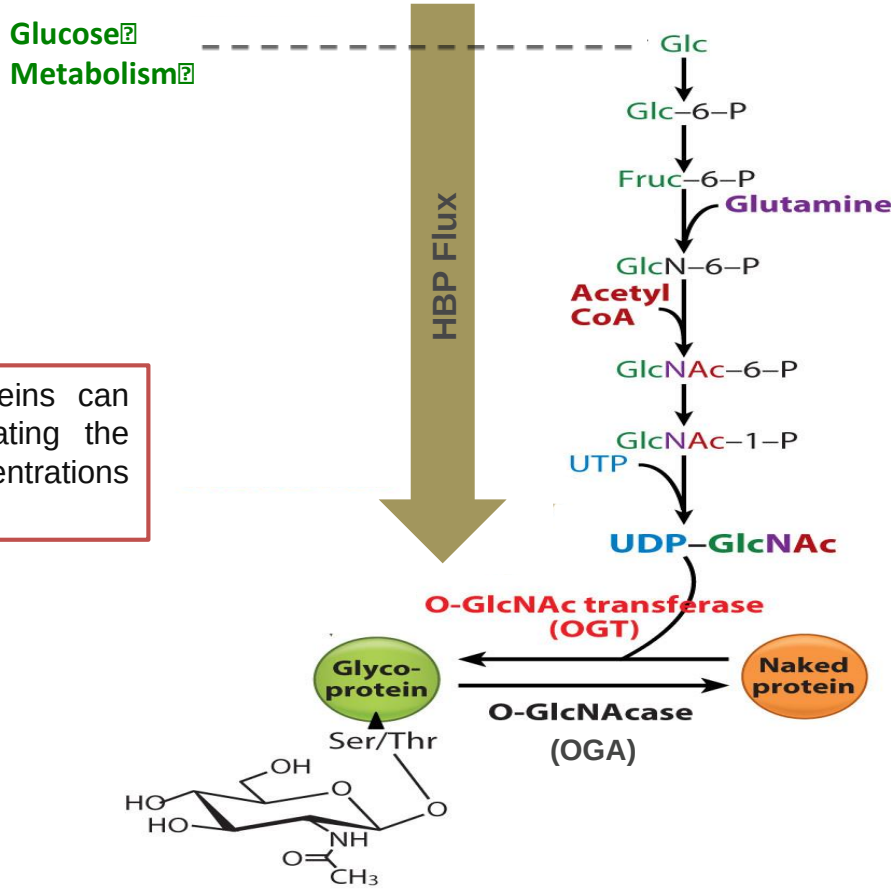
GRK 1657

What is O-GlcNAcylation?

- A dynamic and reversible post-translational modification
- N-acetyl-glucosamine (GlcNAc) is attached to the oxygen atom of specific serine/threonine residues in proteins.
- O-GlcNAcylation levels are dependent on the nutrient-responsive hexosamine signaling pathway



O-GlcNAcylation of proteins can be increased by incubating the cells with high concentrations of glucose



- DNA damage induces O-GlcNAcylation
- O-GlcNAcylation plays an important role in the repair of DSBs

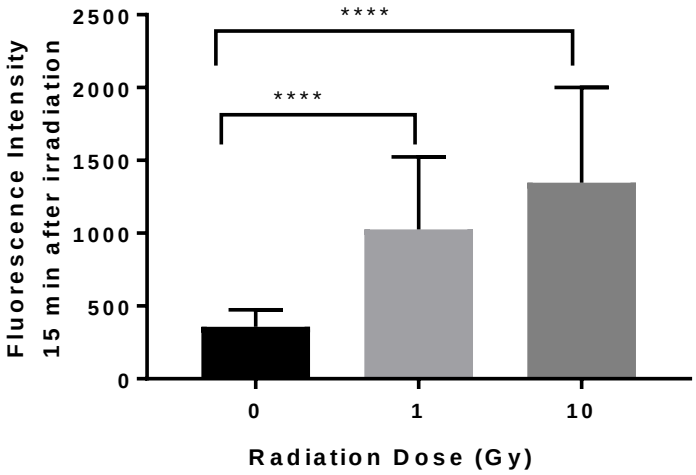
Modified from Hart *et al*, 2011

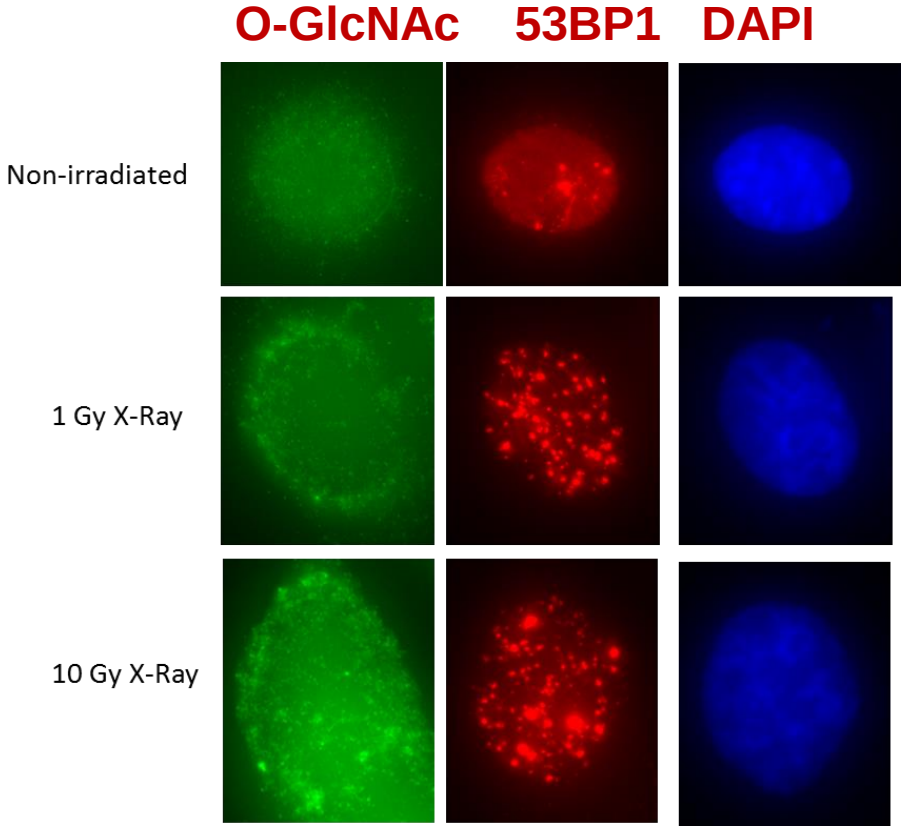
Questions adressed in this work;

- Does PTM O-GlCNAc influence the repair of X-ray induced DSBs?
- Is the repair modulation by the PTM O-GlcNAc depending on damage complexity?

Influence of X-irradiation on O-GlcNAc modification

O-GlcNAc modification 15 min after IR





CTD110.6: Anti-O-GlcNac Antibody

The cellular O-GlcNAc levels increase in response to X-irradiation.

Effects of O-GlcNAcylation on X-irradiation induce *γH2AX* foci

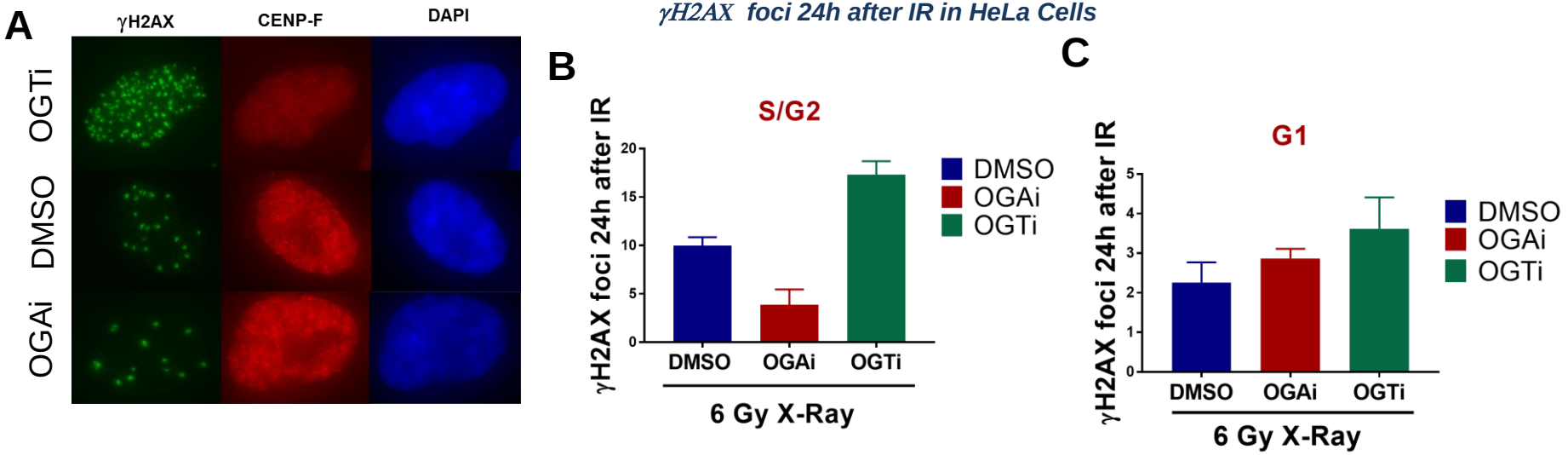


Figure: DNA DSBs repair in O-GlcNAc modulated HeLa CCL2 cells after X-Ray radiation. ɣH2AX foci were quantified in DMSO (control), OGTi or OGAi treated HeLa cells in G1 and S/G2 phases 24 h after irradiation. Manipulating O-GlcNAcylation did not influence the number of induced gH2AX foci in G1 and S/G2 phase cells. **A:** ɣH2AX foci were imaged 24 h after irradiation in O-GlcNAc modulated HeLa cells. **B-C:** The averaged number of radiation-induced ɣH2AX foci per nucleus are shown in G1 and S/G2 phase. At least 50 nuclei were counted for each condition. The data represent the mean ± SEM of 2 independent experiments.

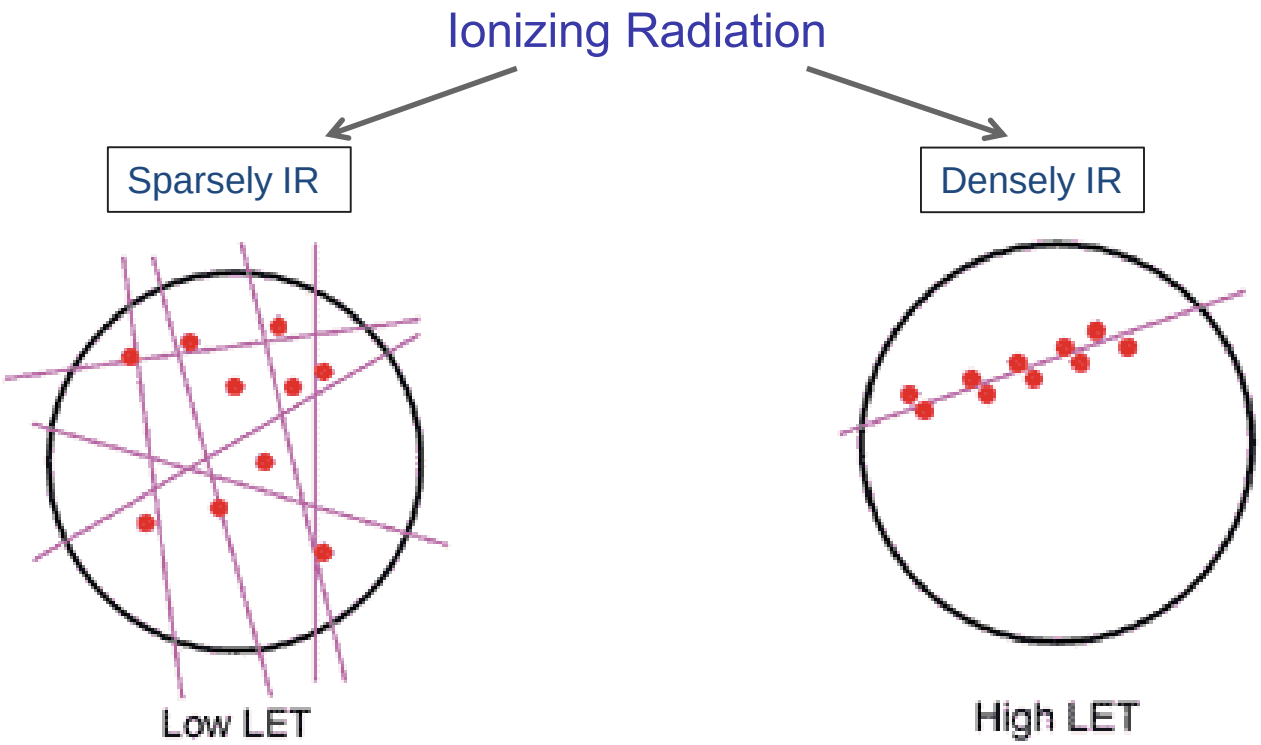
- Loss of O-GlcNAcylation impaired DSBs repair capacity.
- Promoting O-GlcNAc modification improved DNA DSBs repair.

Questions adressed in this work;

- Does PTM O-GlCNAc influence the repair of X-ray induced DSBs?

- Is the repair modulation by the PTM O-GlcNAc depending on damage complexity?

Sparsely and densely ionizing radiation and its biological effects



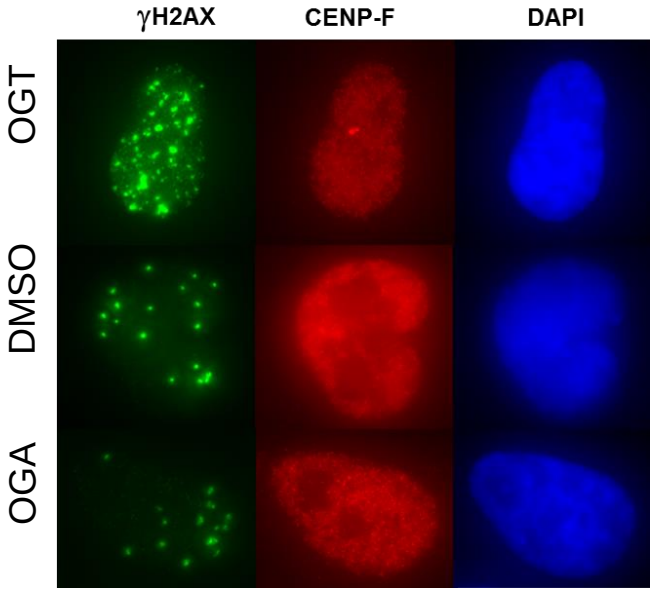
- X- and γ-rays are sparsely-ionizing radiation.
- Energy is distributed homogenously
- The energy are deposited to the entire irradiated area
- Less complex DNA damage sites

- Protons, α-radiation and heavy ions are densely- ionizing radiation.
- Energy is distributed non-homogenously
- The energy is transferred along its paths
- Many complex DNA double strand breaks- leads to cell death

Maren Herrlitz, PhD Thesis,2014

Effects of O-GlcNAcylation on repair kinetics of DSBs induced by C-ion

HeLa cells were irradiated with C-ion (170 keV/µm)



Repair kinetics of DNA DSBs

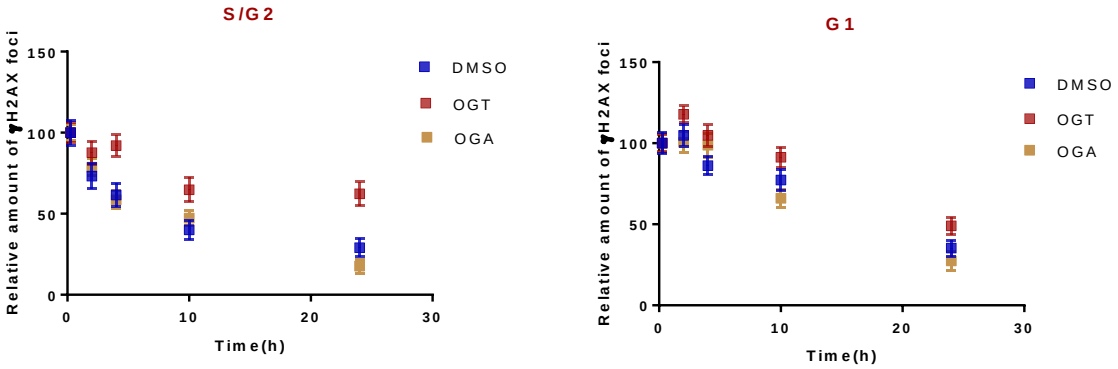


Figure: DNA DSBs repair in O-GlcNAc modulated HeLa CCL2 cells after C-ion irradiation. γH2AX foci were quantified in DMSO (control), OGTi or OGAi treated HeLa cells in G1 and S/G2 phases. **A:** γH2AX foci were imaged 24 h after irradiation in O-GlcNAc modulated HeLa cells. **B-C:** The relative amount of radiation-induced γH2AX foci are shown in G1 and S/G2 phase. At least 50 nuclei were analysed for each condition. The data represent the mean ± SEM of 1 experiment.

- Elevated level of O-GlcNac increased the repair capacity of C-ion induced DNA DSBs.
- Inhibition of O-GlcNAc decreased the capacity to repair C-ion induced DSBs.

Influence of O-GlcNAcylation on heavy-ion radiation sensitivity

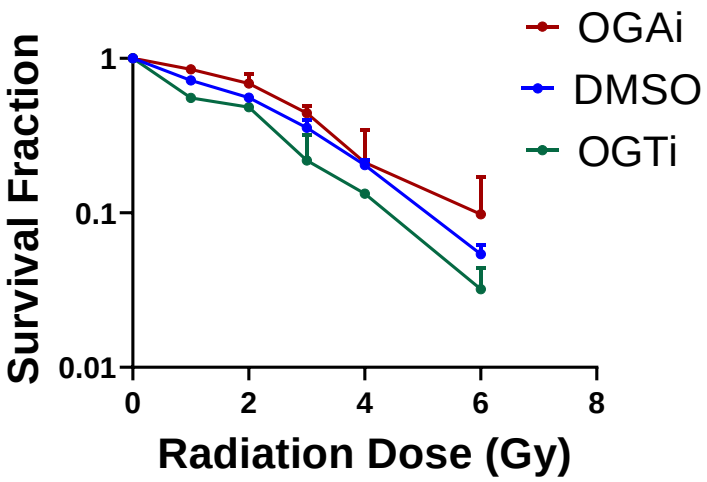


Figure: Clonogenic survival of HeLa cells after treatment OGAi, OGTi or DMSO. The data represent the mean± SD of 2independ entexperiments.

O-GlcNac inhibition leads to increased radiation sensitivity.

CONCLUSION and Outlook

- Cellular O-GlcNAc levels increased after X-irradiation.
- Targeting O-GlcNAcylation modulates DNA damage response.
- Effects of O-GlcNAcylation on the repair of DNA DSBs is identical between X-Rays and C-ion.

Future Direction

Since the O-GlcNAc dependent repair modulation was found mainly in S/G2 cells, we will adress, if the observed effects are based on HR (homologous recombination).