USP9X inhibition enhances radiosensitisation of head and neck cancer cells in response to high-LET radiation by destabilizing centrosome proteins

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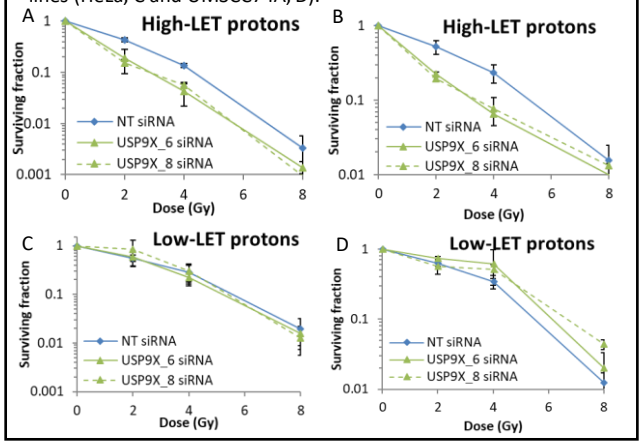
The mechanisms involved in the impact of IR of higher linear energy transfer (LET) on cell biology are still unknown. We have recently performed siRNA screening to identify deubiquitylating enzymes that control cell survival specifically in response to high-LET α-particles and protons, in comparison to low-LET X-rays and protons[1,2]. From this screening, we have thoroughly validated that depletion of the ubiquitin-specific protease 9X (USP9X) in HeLa and oropharyngeal squamous cell carcinoma significantly decreases survival of cells after exposure to high-LET radiation, whilst no effect was observed after low-LET treatment. While USP9X inhibition does not interfere with DNA damage repair nor does it induce apoptosis or senescence post-irradiation, we observed that its depletion destabilizes key centrosome proteins (CEP55 and CEP131) causing centrosome amplification and ultimately promoting cell death in response to high-LET protons.

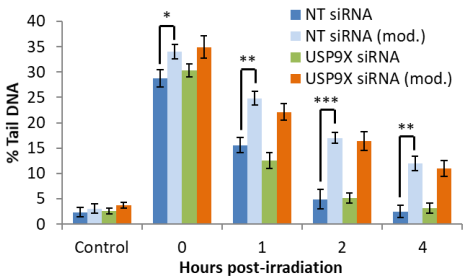
1. USP9X modulates cell survival in response to high-LET IR

USP9X siRNA (labelled sequences 6 and 8) led to reduced survival in response to high-LET protons in HeLa (A) and in UMSCC74A (B), without affecting cell survival after low-LET IR in both cell lines (HeLa, C and UMSCC74A, D).



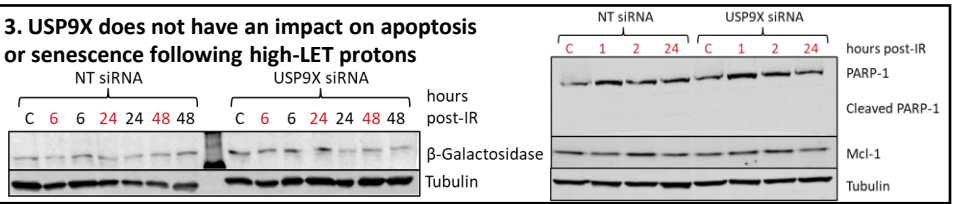
2. USP9X does not interfere with CDD repair following high-LET protons

On depletion of USP9X, we observed that the efficiency of DNA double strand break (DSB) as well as complex DNA damage (CDD) repair in comparison to NT control siRNA treated cells was not significantly different.



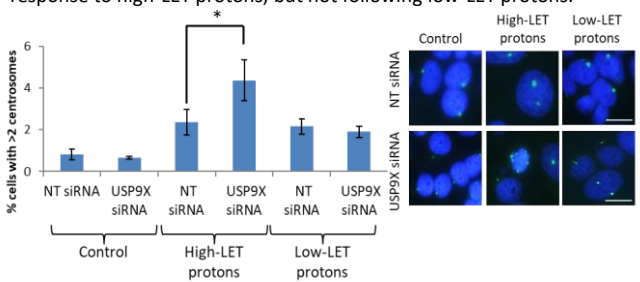
Dark blue and green bars indicate DSBs. Light blue and orange bars (labelled Mod.) indicate levels of CDD by using APE1, OGG1 and NTH1 recombinant enzymes post-cell lysis.

3. USP9X does not have an impact on apoptosis or senescence following high-LET protons



4. USP9X inhibition causes centrosomal amplification after high-LET protons

In USP9X depleted cells, centrosome amplification increases by 1.9-fold compared to NT control siRNA treated cells specifically in response to high-LET protons, but not following low-LET protons.



CONCLUSIONS. Effective radiosensitisation strategies for improving the outcome of HNSCC patients are actively being sought[3]. Our data suggest that USP9X is essential for chromosome stability and cell survival, particularly following high-LET radiation, and therefore a possible therapeutic target for enhancing HNSCC radiosensitivity under these conditions.

REFERENCES.

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