Complex DNA double strand breaks induced by heavy ions require resection for their repair*

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DNA double strand breaks (DSBs) represent harmful lesions, which can jeopardize genomic stability and even can be lethal. Therefore, different repair pathways exist to remove them. The pathway choice is influenced by the complexity of the lesions, which are increasingly difficult to repair with increasing complexity.

Inducing DSBs of a wide range of complexity by irradiating mammalian cells with X-rays or accelerated ions of different velocity and mass, we found that with increasing complexity DSBs become increasingly resected in all cycle stage [1]. To find out whether the observed resection of complex DSBs is relevant for their repair, we measured DSB repair kinetics of ion- and X-ray induced DSBs in cells that were impaired for resection by depletion of the resection factor CtIP (Fig. 1).

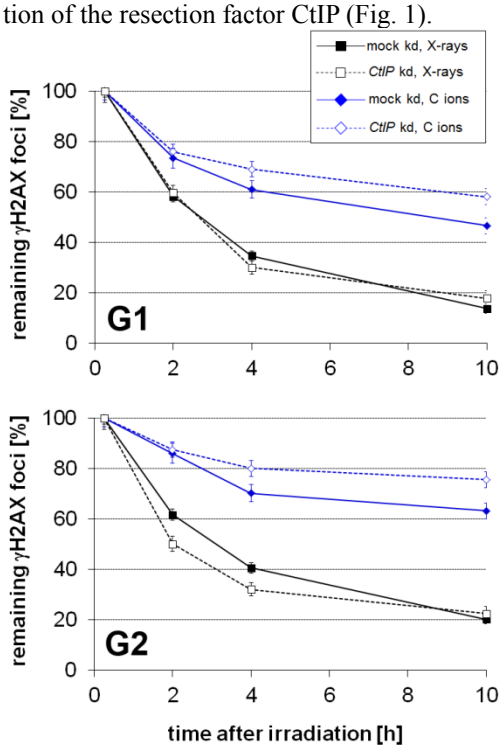

Figure 1#: Repair of complex DSBs is resection dependent. Human, immortalized fibroblasts were depleted or mock depleted for CtIP and irradiated with 1.3 Gy X-rays or carbon-ions (LET 170 keV/µm). Aphidicolin treatment prevented G1-cells from moving on to G2 and allowed to discriminate S-phase cells. DSB-repair kinetics were measured by the γH2AX (DSB marker)-foci assay [2] in G2- (CENP-F positive) and G1-cells (CENP-F negative).

We clearly see impaired DSB repair upon C-irradiation, which is due to the increased lesion complexity and was described earlier. CtIP depletion impairs repair of C-ion induced complex DSBs in G2- and G1-cells, but not of X-ray induced DSBs of lesser complexity. Thus, abolished resection does not allow repair of complex DSBs to switch to resection independent pathways suggesting that the pathway choice of these lesions is forced towards resection-dependent repair. Taken together, resection plays an important role within the repair of complex DSBs.

Resection of simple DSBs is cell cycle regulated via 53BP1/RIF1 and BRCA1/CtIP, where the former prevent resection and are replaced by the latter to allow resection in G2-cells [3]. As complex DSBs are resected even in G1-cells we started to analyze how the above factors are involved in the resection regulation of complex DSBs. Therefore, in a first step we studied whether RIF1 is recruited to ion-induced DSBs. We clearly see that RIF1 is recruited to Xe-induced DSBs in G1- and S/G2-cells (Fig. 2); data analyses revealed that almost all G1- and half of S/G2-phase cells are RIF1 positive. Future studies will reveal how resection of ion-induced DSBs is regulated.

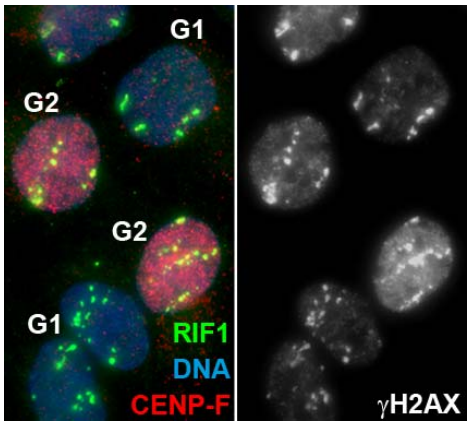

Figure 2: RIF1 is recruited to ion-induced DSBs in G1 and G2-cells. Human osteosarcoma cells were irradiated with Xe-ions in a low angle (9,000 keV/µm; 11.4 MeV/u; 3 x 10^6 p/cm^2) and fixed 1 h after irradiation. CENP-F (red): cell cycle marker, γ-H2AX (white): DSB marker.

References

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