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The histone H2AX does not inhibit resection of DNA double strand breaks induced by heavy ions

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The histone H2A variant H2AX plays an important role in the repair of DNA double strand breaks (DSBs) within eukaryotes. Very rapidly upon DSB induction this H2A variant becomes phosphorylated at Ser139 [1]; H2AX phosphorylated at this site is named γH2AX. It serves as a platform for several DSB repair factors [reviewed in 2] and further is thought to stabilize the loose ends of a DSB [3]. In addition, γH2AX controls the end processing of recombination activating gene (RAG) endonuclease induced DSBs within V(D)J recombination, which takes place in the G1 cell cycle phase of lymphocytes. Absence of γH2AX within this process allows CtIP-mediated DSB resection [4]. Hence, γH2AX prevents resection within the V(D)J recombination and it is conceivable that resection of genotoxic caused DSBs is as well under the control of γH2AX [4], especially in the cell cycle phase G1 when DSB resection would lead to genomic instability.

Studies here at GSI clearly demonstrated that CtIP-driven resection of heavy ion induced DSBs occurs not only in the late S and G2 cell cycle phase but in G1 as well [5] sustaing the notion that resection of ion induced DSBs in G1 is not inhibited by γH2AX. This is further supported by the observation that in ion irradiated confluent human fibroblasts (AG1522D) (figure 1), where 98 % of cells were in the G1/G0 cell cycle phase, most DSBs are resected and the resection marker pRPA always colocalizes with γH2AX at ion induced DSBs. There was not one cell where RPA was recruited to a break site where H2AX was not phosphorylated. This was observed in three independent experiments (2x ^{238}U irradiation, LET: 15 000 keV/µm, fluence: 3 x 10^6 p./cm^2; 1x ^{207}Pb irradiation, LET 13500 keV/µm, fluence 3 x 10^6 p./cm^2). Thus, γH2AX seems not to inhibit DSB resection of heavy ion induced lesions.

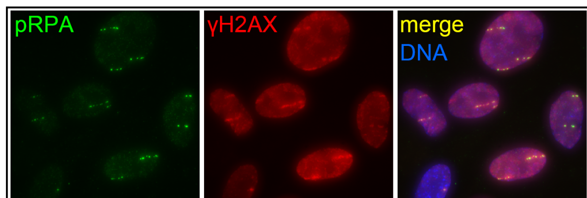

Figure 1: **Resection of ion induced DSBs is never observed independently of γH2AX.** Confluent human fibroblasts were irradiated with ^{207}Pb ions (LET: 13500 keV/µm; fluence: 3 x 10^6 p/cm^2) and fixed 1 h after irradiation. Cells were immuno-stained for γH2AX (DSB marker) and pRPA (resection marker). DNA was visualized by DAPI staining.

In order to further characterize whether or not DSB resection in G1 is controlled by H2AX and in particular by γH2AX we studied DSB resection in H2AX deficient cells. Murine H2AX -/- and wild-type cells were ^{12}C ion irradiated (LET: 170 keV/µm; fluence: 3 x 10^6 p/cm^2) and immuno-stained against RPA (resection marker) and 53BP1 (DSB marker) 1 h after irradiation. If γH2AX were inhibiting ion induced DSB resection in the G1 cell cycle phase one would expect that the H2AX deficient cells show an increase in resected DSBs. However, H2AX deficient and wild type cells both show about 20 % of resected DSBs. A different cell cycle distribution cannot be responsible for the obtained result as approximately 50 % of both populations were in the G1 cell cycle phase (determined by flow cytometry). As H2AX deficiency did not influence the fraction of resected ion induced DSBs in G1 we conclude that γH2AX indeed does not inhibit resection of heavy ion induced DSBs in cells within the G1 cell cycle phase.

Taken together, from these observations we conclude that H2AX most likely has no function in controlling end resection of genotoxic-induced DSBs. Its influence on DSB resection might, thus, be unique to V(D)J recombination.

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