Abstract “Global chromatin changes induced by altered tonicity interferes with DNA damage response signaling and DNA double-strand break repair”

The results of our experiments reveal that global changes in chromatin structure achieved by hypotonic or hypertonic treatment have severe consequences on DDR signaling and DSB repair and thereby endanger genomic stability.

Chromatin relaxation by itself results in transient arrest of cells in G2-phase, which might be due to ATM activation. However, in response to IR DDR signaling is reduced in terms of pATM, γH2AX and 53BP1 foci formation. Surprisingly, we still see a strong G2-checkpoint response upon DSB induction, which is dependent on ATR as well as on ATM. Further investigation of upstream signaling of ATM will help to understand these diverse responses mediated by ATM. Since we observe functional DNA end resection in globally relaxed chromatin, an ATR mediated checkpoint response is promoted. However, DSB repair by the main repair pathways is disturbed, as we see less cNHEJ and especially HRR. Also altEJ fails to backup cNHEJ and HRR, but SSA is three-fold increased upon hypotonic treatment. As this is seen in experiments utilizing reporter cell lines, it would be interesting to investigate the response of SSA to chromatin relaxation on IR-induced DSBs.

While impairments of faithful DSB repair are seen and in addition only mutagenic DSB repair is functional, the modest effects found in survival are unexpected. However, as continuous hypotonic treatment is toxic to the cells, the adaptation of the protocol to transient treatment explains the small radiosensitizing effect observed. DDR signaling experiments and also measurements of CCP show that effects of global chromatin relaxation mediated by hypotonic treatment are completely reversible.

Global chromatin condensation achieved by hypertonic treatment arrests cells in G1-, G2- and M-phase, while it enhances DDR signaling in terms of ATM activation and H2AX phosphorylation in response to IR. However, DSB repair efficiency of all investigated repair pathways is compromised (cNHEJ) or completely abrogated (HRR, altEJ and SSA). This might be explained by nonfunctional DNA end resection upon chromatin condensation, which is necessary for HRR, altEJ and SSA. However, activation of checkpoints may help to maintain genomic stability although DSB repair is inhibited. Chromatin condensation and also DDR signaling is restored when cells

are re-incubated in normal cell culture medium, which explains the moderate effects of hypertonic treatment on radiosensitization to killing observed.

Collectively, global changes in chromatin massively perturb responses to DNA damage and risk genomic integrity. The action of hypotonic and hypertonic medium in altering chromatin structure is rather unspecific. Thus, more specific treatments like inhibitors of proteins influencing chromatin structure or genetically modified cell lines with loss-of-proteins that are known to facilitate, for example, chromatin condensation will help to further elucidate the role of chromatin structure in DDR signaling, DSB repair and DSB repair pathway choice.