The Role of Homologous Recombination Repair in the processing of G2-Chromosomal Breaks and maintenance of G2-Checkpoint

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DNA double strand breaks are potent inducers of cell cycle checkpoints, DNA damage response (DDR) signalling and repair pathways. Ionizing radiation (IR) efficiently induces DSBs. G2-M checkpoint activation delays/stops cell division to facilitate repair of these lethal lesions. According to the classical view proteins of PI3K kinase family ATM and ATR have been shown to be the key players in the activation of this checkpoint. In higher eukaryotes, two main repair pathways named NHEJ and HRR are responsible for the repair of DSBs.

For many years, HRR has been considered to be a minor pathway of DSB repair in higher eukaryotes. This opinion is based on the fact that none of the cell lines deficient in important HRR genes displays obvious DSB repair defects although they are clearly radiosensitive to killing. It is therefore thought that in higher eukaryotes IR induced DSB repair is practically exclusively undertaken by the D-NHEJ pathway; on the other hand the role of HRR is only evident on the repair of I-SceI induced site directed DSBs in integrated genomic loci. Thus, how HRR contributes to the repair of IR induced DSBs remains unknown.

In the work presented here, we study the repair of IR induced DSBs through their transformation into G2-chromosomal breaks. Our results show the direct involvement of HRR in the repair of a subset of IR induced DSBs which are associated with the formation of G2-chromosomal breaks. The D-NHEJ repair pathway does not seem to play a role in the repair of these breaks.

Our results also demonstrate that HRR is a key player in the activation and maintenance of the G2-checkpoint. We employed flow cytometry based determination of mitotic index after exposure to 1Gy X-rays using phosphorylated histone-H3 as a marker. Chinese hamster cells deficient in RAD51 paralogs RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 show a remarkable defect in G2-checkpoint activation, while cells deficient in NHEJ components KU80, DNA-PKcs and XRCC4 show strong G2- checkpoint activation when compared to the wild type cells.

This is, to our knowledge, the first report implicating HRR in the repair of a subset of DSBs with the potential of forming chromosome breaks, as well as in the development and maintenance of DNA damage induced cell cycle checkpoints.