Abstract

Double strand breaks (DSBs) are the most severe form of DNA lesions that are repaired in higher eukaryotes by DNA PK-dependent non-homologous end-joining (D-NHEJ), homologous recombination repair (HRR) or alternative non-homologous end-joining (alt-EJ). From all DSB repair pathways, only HRR has a build-in mechanism to completely restore the DNA sequence around the breaks, while D-NHEJ is frequently associated with errors. Alt-EJ is commonly associated with chromosomal translocations, large deletions and in general genomic instability. However, it is still not known how increasing DSB complexity affects DSB repair pathway choice. To address this question, two different approaches were introduced in the present thesis.

In the first approach we studied reversion in the Chinese hamster *hprt* mutants, SPD8 and SP5. Using this biological model system, we could study the role of DSB complexity in reversion induction and the contribution of DSB repair pathways to the correction events induced. It is particularly useful that reversion in the SPD8 and SP5 mutant require homologous and non-homologous recombination, respectively. Reversion results generated after exposure of SPD8 and SP5 cells to low and high-linear energy transfer (LET) ionizing radiation showed only limited induction of reversions that was difficult to interpret as deriving from DSBs of different complexity.

Therefore, we specifically induced DSBs within the *hprt* gene by applying the RNA guided nuclease technique (CRISPR/Cas9). Our results revealed that a single DSB generated directly in the exon flanking intron region of the mutated region in SPD8 and SP5 cell lines efficiently triggers reversion, while DSBs in the surrounding region triggers reversion markedly less efficiently.

Furthermore we could document that DSB clusters impair the correction mechanisms in SPD8 and SP5 cell lines. DSB clusters are apparently more difficult to handle and compromise repair events supporting reversion. The DSB repair pathway analysis carried out using specific inhibitors revealed distinct repair pathway contributions in the SPD8 and SP5 mutants. D-NHEJ and partially HRR facilitated the homologous recombination events supporting reversion in SPD8 cells, whereas D-NHEJ interfered with the process of non-homologous recombination in SP5 cells. Thus, we could demonstrate that D-NHEJ is not always error prone.

In the second approach we have adapted the forward mutation assay in the Chinese hamster V79 cells and examined the contribution of single DSBs as well as of DSB clusters, and or DSB repair pathways to mutagenesis. We found that the induction of a DSB within intron or intron/exon junctions, and even more pronouncedly in exon regions, enhance mutagenesis, confirming that DSBs is a severe DNA lesion. Increase in DSB complexity in the form of DSB clusters, further increase mutation induction particularly when the DSB cluster removes an entire exon or several exons. They generate less severe effects when generated in introns or intron/exon junctions. The DSB repair pathway investigation revealed a protective role for D-NHEJ at complex DSBs. In contrast alt-EJ contributed predominantly to DSB related mutagenesis at single DSBs.

The exact mechanisms as to how repair pathways coordinate their activities and contribute to genome stability could not be explained conclusively by our experiments. The further elucidation of this highly important aspect of genomic stability will be the focus of future studies in the Institute of Medical Radiation Biology.