Abstract

Sparsely ionizing radiation (IR) deposits energy in spurs and blobs where multiple ionizations occur in a small volume. If generated close to DNA, such events induce multiple lesions within few base pairs (complex lesions) and damage both base and sugar moieties. These energy deposition events are closely confined to the paths of the constituent ionizing particles. The distances between subsequent ionization events vary randomly along the particle path, and decrease as the particle slows down and comes to a halt. Thus, at all ends of electron tracks, where X-ray energy is mainly deposited, excessive local accumulation of ionizations will generate complex DNA damage that is thought to be primarily responsible for the observed adverse cellular effects.

The DNA double strand break (DSB) is the simplest form of a complex lesion, generated when two ionizations, or radiation-induced radicals, in close proximity disrupt the sugar phosphate backbone of the two DNA strands. Further complexity, and thus also increased risk for biological consequences, is generated when additional lesions occur near the DSB. Such complex damage is presently intensively studied and is implicated in the biological effects of high LET radiation. Another dimension of complexity and a particularly high risk for biological consequences is generated when two (or more) DSBs are induced in close proximity, as they may completely destabilize chromatin and may cause DNA losses that cannot be dealt by available DNA repair pathways. Although this form of complex DNA lesion has been considered in the past, mechanistic studies able to analyze its biological consequences are lacking.

In vitro manipulation of the plasmid DNA allows the generation of DSBs with different but well-defined ends, usually generated by treatment with appropriate combinations of restriction endonucleases. While these assays recapitulate important aspects of D-NHEJ, several reports show that they fail to reproduce the marked reduction in DNA end joining efficiency of genomic DSBs observed in a variety of D-NHEJ mutants, suggesting that they mainly reflect the function of B-NHEJ. In preparation for the above mentioned studies on complex lesions we also examined the effect of the transfection method on repair pathways selection. We discovered that while electroporation generates plasmid end joining results in the different mutants closely reflecting rejoining of genomic DSBs, plasmid lipofection-generated results showing a nearly wild type plasmid end joining

efficiency in D-NHEJ mutants. We show that the subcellular localization of the transfected plasmid underlies these differences.

With this information at hand, we began studies to test the hypothesis that clustering of DSBs at distances affecting nucleosomal or local chromatin stability is highly lethal for the cell. Rather than mathematically modeling energy deposition and the general features of clustered damage induction and fitting the model to the biological effects measured, here we model the biological lesion in a highly specific way by narrowing down on the candidate lesion and testing for key biological consequences. This approach has the advantage that it allows the characterization of the general properties of radiation lesions causing mutations and cell lethality.

We describe model systems in which DSB clusters are induced in the genome of human tumor A549 cells through enzymatic restriction of I-*Sce*I sites integrated at different combinations and at multiple sites using transposon technology. To address complexity, we generated transposon-based constructs harboring a single and two I-*Sce*I sites in compatible and incompatible orientations separated by 200bp. The vector used also contains the neo^R gene as a selectable marker. The integration of the plasmid at multiple sites is facilitated by co-expression of a hyperactive transposase artificially reactivated from the “Sleeping Beauty” transposon. The number of integration sites is measured in randomly selected G418 resistant clones using Southern blotting and sets are generated with clones harboring 3-12 integration sites for each DSB arrangement.

Using these sets of clones, cell survival by colony formation, as well as chromosomal aberration formation along with other techniques like mitotic index/G2-checkpoint activation, and induction of EGFP-53BP1 foci are studied. The results obtained as part of this thesis show a correlation between lesion complexity and cell killing. Evidence for checkpoint activation and chromosome aberration formation is also obtained, but analysis of these endpoints is hampered by the apoptotic response of the generated system. More work will be necessary to define and improve the potential of this model system for radiation damage.

Key words: **Ionizing radiation, Transposon, Complex lesion, LET, Nucleofection, Lipofection, Chromatin, D-NHEJ and B-NHEJ**.