Processing of Clustered DNA Damage in In-vitro Biopsies of Intact Human Epidermis vs. Cultured Cells Exposed to Low Doses of X-rays

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Clustered damages consisting of bistranded multiple oxidized bases, abasic sites, or strand breaks within a few helical turns of DNA are hallmark damages of ionizing radiation exposure. They can be detected in cells exposed to very low level radiation doses and pose a lethal and mutagenic threat to genomic integrity. Two dimensional human cell culture systems have proven instrumental in beginning to clarify the molecular mechanisms and pathways involved in the processing of clustered DNA damage. Early predictions hypothesized that simultaneous bistranded repair of clustered damages would yield *de novo*, repair generated DNA double strand breaks (DSBs). In studies, our own and those of others, performed at high radiation doses, where clonogenic survival is often poor, *de novo* double strand breaks are indeed generated from the resolution of clusters. However, at low dose radiation exposures, where clonogenic survival is very good, cells avoid DSB generation and use alternate repair or bypass pathways. The repair strategies (which have intact circulatory, neural and immunological components) utilized by intact human tissues for repair of clustered DNA damage are largely unknown and may differ significantly from cells in culture. To investigate this possibility, we have developed a system using intact human skin biopsies to study the processing of clustered DNA damage in a cutaneous tissue environment. Healthy human skin biopsies, obtained from leftover skin from abdominoplasty procedures were exposed to low doses of low linear energy transfer (LET) X-rays, and individual samples were harvested as a function of time after irradiation. Parallel experiments were performed with primary cultures of human skin cells to similar doses of the same radiation. DNA was isolated in agarose plugs from each sample and digested with the 8-base recognition sequence restriction endonuclease Asc I. Samples were either directly electrophoresed and imaged for the quantification of DNA DSBs or further processed with *E. coli* endonuclease IV (Nfo protein) (principal substrates: oxidized and standard abasic sites) or *E. coli* formamidopyrimidine-DNA glycosylase (Fpg protein) (principal substrates: oxidized purines) for detection of abasic or oxidized purine clustered DNA damages respectively**.** Comparisons of the repair kinetics of abasic and oxypurine clusters between cultured cells and intact human epidermis will be presented.

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